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THE SYNTHESIS OF COMPOUNDS  
RELATED TO MYCELIANAMIDE

A THESIS

Presented to  
The Faculty of the Graduate Division  
by  
Richard Kirven Brantley

In Partial Fulfillment  
of the Requirements for the Degree  
Doctor of Philosophy in the  
School of Chemistry


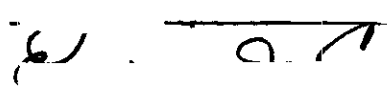

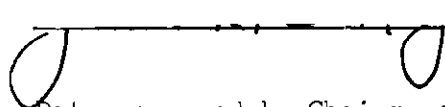
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## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS . . . . .	ii
LIST OF FIGURES . . . . .	v
SUMMARY . . . . .	vi
Chapter	
I. INTRODUCTION . . . . .	1
II. DISCUSSION OF RESULTS . . . . .	13
III. EXPERIMENTAL . . . . .	44
Instrumentation . . . . .	44
Attempts to Prepare 1,4-Dihydroxy-2,5-diketopiperazine from 2,5-Diketopiperazine by Oxidation . . . . .	44
Attempt to Prepare 1,4-Dihydroxy-2,5-diketopiperazine by Ring Closure Methods . . . . .	47
Reduction of $\alpha$ -Nitroesters to $\alpha$ -Hydroxylamino Esters and Attempts to Cyclize These Compounds into 1,4-Dihydroxy- 2,5-diketopiperazine . . . . .	48
3-Methyl-2,5-diketopiperazine . . . . .	50
3,6-bis(p-Acetoxybenzylidene)-2,5-diketopiperazine . . . . .	52
p-Acetoxybenzaldehyde . . . . .	53
3-(p-Acetoxybenzylidene)-1,4-diacetyl-6-methyl-2,5-dike- topiperazine . . . . .	53
3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine . . . . .	
Attempts to Prepare Tetrahydro-2-(4-hydroxybenzyl)- 5-methyl-4-oxoglyoxaline-2-carboxylic Acid from 3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine . . . . .	56
Ethyl $\gamma$ -Bromobutyrate . . . . .	57
Reactions of Ethyl $\gamma$ -Bromobutyrate with Methyl Magnesium Halides . . . . .	58
4-Methyl-1,4-dihydroxypentane . . . . .	59
4-Methyl-1,4-dibromopentane . . . . .	60
Dehydrohalogenation of 4-Methyl-1,4-dibromopentane with Potassium Benzoate . . . . .	62

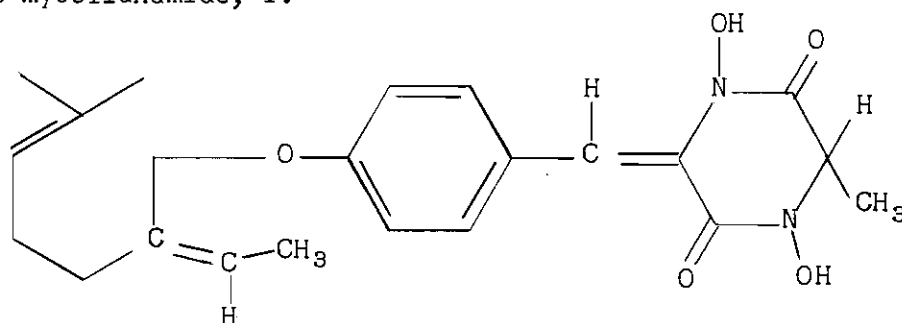
Dehydrohalogenation of 4-Methyl-1,4-dibromopentane with Alcoholic Potassium Hydroxide . . . . .	63
Alkylation of Ethyl Acetoacetate with 1-Bromo-4-methyl- 3-pentene . . . . .	64
Reduction of Ethyl 2-Acetyl-6-methyl-5-heptenoate with Sodium Borohydride . . . . .	66
Dehydration of Ethyl 2-(1-Hydroxyethyl)-6-methyl- 5-heptenoate . . . . .	67
Separation of <u>cis</u> - and <u>trans</u> -Ethyl 2-Ethylidene- 6-methyl-5-heptenoate . . . . .	71
Attempts to Isomerize the Ethylidene Double Bond of Ethyl 2-Ethylidene-6-methyl-5-heptenoate . . . . .	72
Reduction of <u>cis</u> - and <u>trans</u> -Ethyl 2-Ethylidene-6-methyl- 5-heptenoate with Lithium Aluminum Hydride . . . . .	73
Geranyl Bromide . . . . .	74
1-Bromo-2-ethylidene-6-methyl-5-heptene . . . . .	75
<u>p</u> -Geranyloxybenzoic Acid . . . . .	77
3-( <u>p</u> -Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine. . . . .	79
3-[ <u>p</u> -(2-Ethylidene-6-methyl-5-heptenoxy)benzylidene]- 6-methyl-2,5-diketopiperazine . . . . .	80
Mycelianamide . . . . .	82
Reduction of Mycelianamide . . . . .	82
FIGURES . . . . .	84
LITERATURE CITED . . . . .	89
VITA . . . . .	91

## LIST OF FIGURES

Figure		Page
1.	Infrared Spectrum of 3-( <u>p</u> -Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine . . . . .	84
2.	N.M.R. Spectrum of 3-( <u>p</u> -Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine . . . . .	84
3.	Infrared Spectrum of 3-[ <u>p</u> -( <u>trans</u> -Ethylidene-6-methyl-5-heptenoxy)benzylidene]-6-methyl-2,5-diketopiperazine . .	85
4.	N.M.R. Spectrum of 3-[ <u>p</u> -( <u>trans</u> -2-Ethylidene-6-methyl-5-heptenoxy)benzylidene]-6-methyl-2,5-diketopiperazine . .	85
5.	Infrared Spectrum of 3-[ <u>p</u> -(2- <u>cis</u> -Ethylidene-6-methyl-5-heptenoxyl)benzylidene]-6-methyl-2,5-diketopiperazine. .	86
6.	N.M.R. Spectrum of 3-[ <u>p</u> -(2- <u>cis</u> -Ethylidene-6-methyl-5-heptenoxyl)benzylidene]-6-methyl-2,5-diketopiperazine. .	86
7.	N.M.R. Spectrum of Mycelianamide . . . . .	87
8.	Infrared Spectrum of Mycelianamide . . . . .	87
9.	Infrared Spectrum of the Reduction Product of Mycelianamide . . . . .	88
10.	N.M.R. Spectrum of the Reduction Product of Mycelianamide . . . . .	88

## SUMMARY

The original aim of this research was the synthesis of the mold metabolite mycelianamide, I.



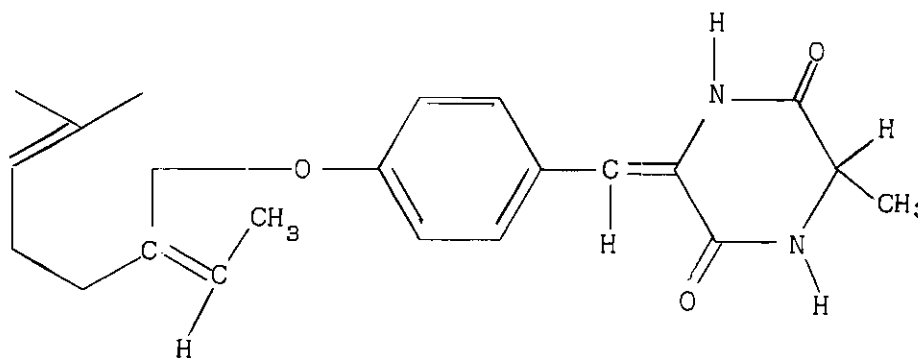
I

This synthesis can be resolved into three separate parts: the heterocyclic portion, the aromatic portion, and the terpenoid portion. The aromatic portion is a residue of p-hydroxybenzaldehyde and offered no synthetic difficulty. No established route for the synthesis of a heterocyclic system similar to the one in mycelianamide was found by a survey of the literature. Since a synthetic route to this type of heterocyclic compound was needed for the synthesis of mycelianamide, the first laboratory work was directed toward the synthesis of 1,4-dihydroxy-2,5-diketopiperazine. Three basically different approaches were tried. The first was direct oxidation of 2,5-diketopiperazine. The second involved ring closure methods utilizing hydroxylamine and  $\alpha$ -halo acid halides. The third approach proceeded by the reduction of



$\alpha$ -nitroesters to  $\alpha$ -hydroxylamino esters and subsequent bimolecular condensation of these compounds. None of these approaches was successful.

Because the 1,4-dihydroxy-2,5-diketopiperazine ring system appeared to be relatively inaccessible, the aim of the research was changed to the synthesis of deoxymycelianamide, II.



II

Deoxymycelianamide is reported to be derived from mycelianamide by mild reduction.

The heterocyclic portion of deoxymycelianamide, 3-methyl-2,5-diketopiperazine was synthesized first. Chloroacetylalanine ethyl ester was prepared by low-temperature acylation of alanine ethyl ester with chloroacetyl chloride in ether solution in the presence of aqueous sodium hydroxide. The chloroacetylalanine ethyl ester was cyclized to 3-methyl-2,5-diketopiperazine by heating it with methanolic ammonia under pressure.

The 3-methyl-2,5-diketopiperazine was condensed with p-acetoxybenzaldehyde by heating the two compounds in the presence of acetic

anhydride and sodium acetate. The product, which was obtained in low yield, appeared to be a mixture of di- and tri-acetyl-3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine. The acetyl groups were removed by the action of aqueous-ethanolic ammonia at room temperature. This yielded the desired 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine. This compound can be recognized as being the aromatic and heterocyclic portion of deoxymydelianamide, II.

The terpenoid portion of deoxymycelianamide, mycelianol, resembles many naturally occurring, available compounds, but does not appear to be easily prepared from these materials by synthetic manipulations. The synthesis of mycelianol started with the addition of two moles of methyl magnesium iodide to butyrolactone. The resulting diol, 1,4-dihydroxy-4-methyl pentane, was converted to 1,4-dibromo-4-methylpentane by the action of hydrogen bromide. This dibromide was selectively dehydrohalogenated by potassium benzoate in acetic acid solution to yield a mixture of olefinic bromides, 1-bromo-4-methyl-3-pentene and 1-bromo-4-methyl-4-pentene. 1-Bromo-4-methyl-4-acetoxypentane was isolated as a by-product in this reaction. The attempted separation of the isomeric olefinic bromides was unsuccessful; therefore, it was necessary to employ this mixture for the remainder of the synthesis. The mixture of olefinic bromides was used to alkylate ethyl acetoacetate. Ethyl 6-methyl-5-heptenoate was isolated using one procedure for the alkylation and the desired ethyl 2-acetyl-6-methyl-5-heptenoate using another. The ethyl 2-acetyl-7-methyl-5-heptenoate was reduced with

sodium borohydride to the desired ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate. After trying a number of unsuccessful methods, this compound was dehydrated by preparing the p-toluenesulfonate ester and treating it with sodium ethoxide. The resulting ethyl 2-ethylidene-6-methyl-5-heptenoate was found by N.M.R spectroscopy to consist of approximately equal amounts of the cis and trans isomers. These isomers were separated by alumina chromatography. The respective cis and trans  $\alpha,\beta$ -unsaturated esters were reduced with lithium aluminum hydride to cis- and trans-2-ethylidene-6-methyl-5-hepten-1-ols (cis- and trans-mycelianols). 2-Ethyl-6-methyl-5-heptenal was isolated by silicic acid chromatography as a by-product in this reaction. Geraneol, cis-mycelianol and trans-mycelianol were converted to their respective bromides by the action of phosphorus tribromide and pyridine. These bromides were noted to react in the presence of alumina.

p-Geranyloxybenzoic acid was prepared in good yield by alkylating ethyl p-hydroxybenzoate with geranyl bromide in dimethylsulfoxide solution in the presence of an equivalent amount of potassium carbonate. The resulting ester was saponified to the acid. The attempted alkylation of the phenol fragment of deoxymycelianamide, 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine with geranyl bromide in dimethylsulfoxide solution in the presence of potassium carbonate gave only very low yields. When the solvent employed was changed to acetone reasonable yields were obtained. trans-Mycelyl bromide was also used to alkylate 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine and the resulting

3-(p-trans-myceloxybenzylidene)-6-methyl-2,5-diketopiperazine was isolated. Similarly the alkylation of 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine with cis-mycelyl bromide was carried out to yield 3-(p-cis-myceloxybenzylidene)-6-methyl-2,5-diketopiperazine, II.

The identity or nonidentity of the synthetic compound with naturally derived deoxymycelianamide was not established because of the following factors: the unavailability of the naturally derived compound; the naturally derived compound is optically active and the synthetic material is racemic; the synthetic material is a mixture of terminal isopropylidene and vinylidene double bond isomers; and the configuration around the benzylidene double bond is unknown both in the natural and synthetic materials.

The physical properties of an impure authentic sample of mycelianamide were recorded. This material was then reduced with zinc and acetic acid to yield a material which is probably not deoxymycelianamide.

Infrared, ultraviolet, and N.M.R. spectral data were obtained on all compounds prepared. Elemental analyses of most of the new compounds were also obtained.

## CHAPTER I

### INTRODUCTION

Mycelianamide was first encountered by Anslow and Raistrick in 1931 in their work on the isolation of 6-methylsalicylic acid (1). It was mentioned again by Oxford, Raistrick, and Simonart in their communication on fulvic acid, and the same authors described a method for separating it from the accompanying griseofulvin (2,3).

The first publication on the determination of the structure of mycelianamide by Oxford and Raistrick appeared in 1948 (4). This paper described the isolation of mycelianamide from a culture of Penicillium griseo-fulvum Dierckx, the general physical and chemical properties of mycelianamide, and its bacteriostatic action on various microorganisms. The microorganisms tested included several strains of Staph. aureus, Strep. viridans, and B. anthracis. Some of these bacteria were completely inhibited by concentrations as low as 0.002 per cent. The pertinent chemical data given in this paper include the following: the compound, m.p. 170-172°dec. was found to be a colorless, levorotatory, crystalline substance of the empirical formula  $C_{22}H_{28}O_5N_2$ . It was slightly soluble in the usual organic solvents. The compound was very readily degraded by both acids and bases. Mycelianamide demonstrated no basic properties, but was shown to be a very weak acid, soluble in sodium carbonate solution, but not in sodium bicarbonate. A number of insoluble metal salts of mycelianamide were

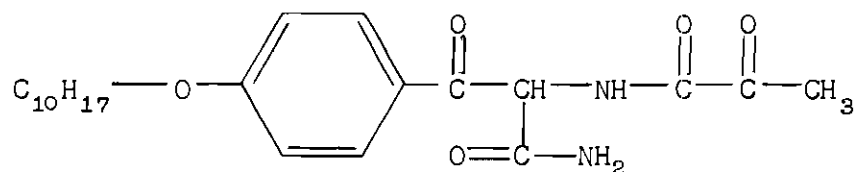
reported. Kuhn-Roth degradation indicated three carbon bound methyl groups were present. Attempts to prepare the usual phenol derivatives failed. Titration of mycelianamide with base indicated it was at least a monobasic acid. However, the sample did not titrate sharply. It gave no reactions for simple carbonyl groups. Upon treatment with ethanolic ferric chloride, it developed an intense, reddish-brown color.

Hydrolysis of a sample of mycelianamide with concentrated hydrochloric acid yielded ammonia and the known compound  $\omega$ -amino-p-hydroxyacetophenone. Hydrolysis by dilute sulfuric acid gave carbon dioxide (1 mole), ammonia (1 mole), and a sweet-smelling, optically inactive, unsaturated hydrocarbon,  $C_{10}H_{16}$  (b.p.  $177^\circ$ ), referred to as mycelene. A small amount of acetaldehyde was also observed. Hydrolysis by dilute base gave ammonia and an optically inactive monobasic acid,  $C_{17}H_{22}O_3$ , I, (m. p.  $118-120^\circ$ ), which showed no phenolic, alcoholic, or ketonic properties. Further hydrolysis of I with dilute sulfuric acid yielded mycelene and p-hydroxybenzoic acid. Hydrolysis by cold dilute ammonia yielded a neutral compound identified as p-myceloxybenzamide. Attempts to obtain this compound by the action of other basic reagents, such as methylamine and alkalis, failed.

Attempts to identify mycelene were inconclusive. Simonsen, after examining a sample of mycelene, suggested that it was a monocyclic hydrocarbon containing two nonconjugated double bonds. Oxford and Raistrick concluded that mycelyl alcohol, the parent alcohol of mycelene, was probably an allylic, acyclic alcohol containing two

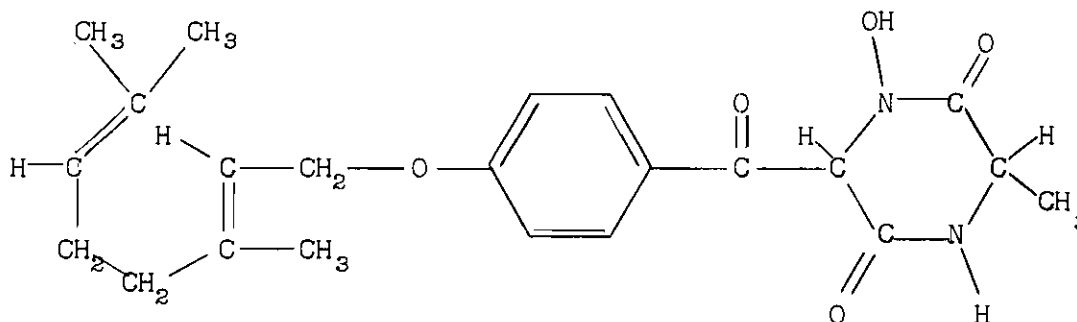
double bonds. An allylic arrangement would account for the ease with which mycelyl alcohol is cleaved from p-myceloxybenzoic acid. Two moles of hydrogen were rapidly absorbed by mycelianamide in the presence of palladium. Ozonolysis of mycelianamide led to the isolation of methylglyoxal and formaldehyde. Mycelyl alcohol, if it has the characteristics suggested by Oxford and Raistrick, can be visualized to undergo cyclization to mycelene under the conditions of the acid hydrolysis.

On the basis of these and other data, Oxford and Raistrick proposed the partial structure (II) as consistent with the data.



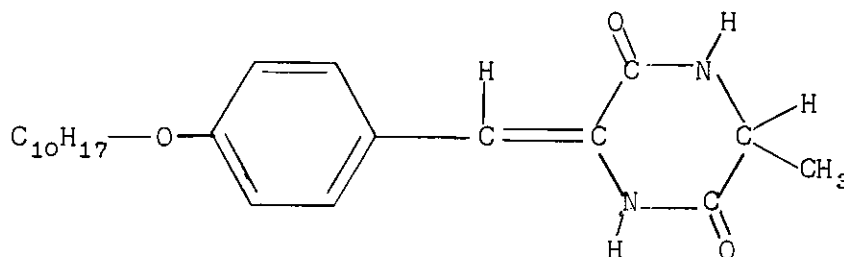
II

Birch, Massy-Westropp and Rickards published a communication of their work on mycelianamide in 1955 (5). They detected the presence of alanine in the acid hydrolysate of mycelianamide. They also established the identity of p-geranyloxybenzoic acid and amide with p-myceloxybenzoic acid and amide obtained from mycelianamide. They therefore proposed Formula III for the structure of mycelianamide. The position of the hydroxyl group was not fixed with certainty. This structure is in accord with most of the data collected by Oxford and Raistrick (4).



III

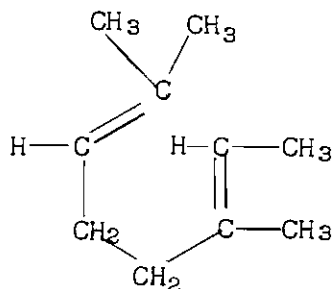
The paper describing the work of Birch, Massy-Westropp and Rickards appeared in 1956 (6). They demonstrated that mycelianamide can be recovered with unchanged optical activity after dissolution in a basic solution. This fact ruled out Oxford and Raistrick's structure II, which would have been expected to racemize in alkaline solution. They next showed that mycelianamide undergoes reduction in the presence of zinc and acetic acid to yield deoxymycelianamide,  $C_{22}H_{28}N_2O_3$ . This compound was insoluble in alkali, gave no ferric test, and had an ultraviolet spectrum very similar to mycelianamide. Isolation of p-hydroxyphenylpyruvic acid and alanine from the acid hydrolysate of deoxymycelianamide support the proposed structure IV for this compound. Infrared spectral evidence was also consistent.



IV



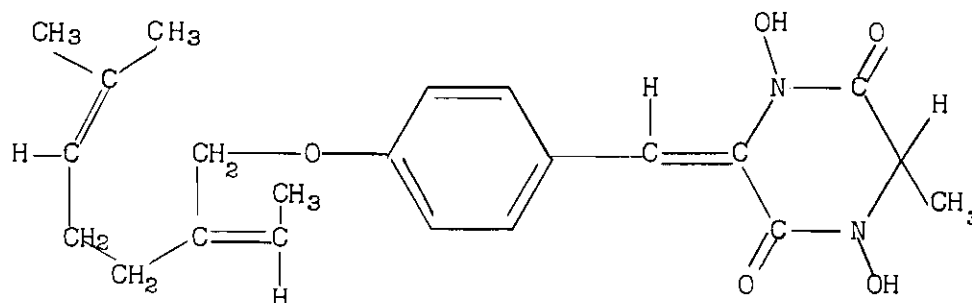
The following work was done on the mycelyl portion of the molecule. The reported identity of p-gernayloxybenzoic acid and amide with p-myceloxybenzoic acid and amide was refuted by differences in their infrared spectra. These compounds demonstrate very similar melting points and the melting points are not depressed by admixture. Careful ozonolysis of p-myceloxybenzamide yielded only acetone and acetaldehyde as products and no levulaldehyde or acid, which would be anticipated from a geranyloxy group. Reduction of mycelianamide with sodium in liquid ammonia yielded a hydrocarbon probably identical with methylgeraniolene, Formula V.



V

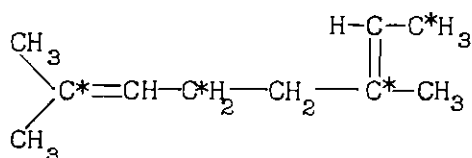
Comparison of the infrared spectrum of this compound with that of an authentic sample obtained by sodium-liquid ammonia reduction of linolool, showed only minor differences. The respective tetrabromides were identical. On the basis of this evidence Birch, Massy-Westropp and Rickards (6) proposed the structure shown on Formula VI for mycleianamide.

The next two publications by Birch and his co-workers dealt with their studies of the incorporation of  $C^{14}$ -labeled acetic, 3-methyl-

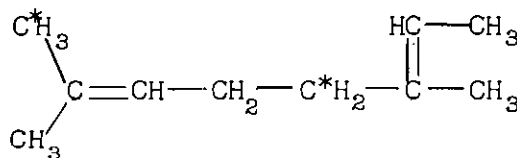


VI

2-butenic, and 3-methyl-3,5-dihydroxypentanoic (mevalonic) acids into the terpenoid portion of mycelianamide (7,8). By careful degradation of the methylgeraniolene, obtained by sodium-liquid ammonia reduction of mycelianamide, the distribution of activity shown in Formula VII was found for  $\text{CH}_3\text{C}^{14}\text{OOH}$  and  $(\text{CH}_3)_2\text{C}=\text{CHC}^{14}\text{OOH}$ . Mevalonic lactone labeled at  $\text{C}_2$  gave rise to the pattern shown in Formula VIII.



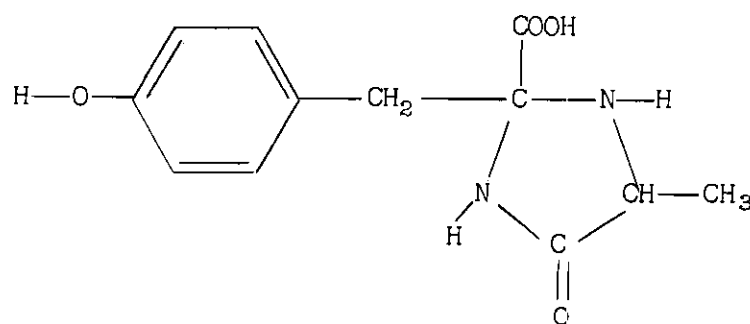
VII



VIII

These data seem to indicate that  $(\text{CH}_3)_2\text{C}=\text{C}^{14}\text{COOH}$  is first degraded to acetate by the mold and then incorporated into the terpene, whereas mevalonic acid is used directly. In all cases the labeled carbon appeared only in the terpenoid portion of the molecule. The remainder of the molecule apparently is composed of a residue each of tyrosine and alanine.

Upon acidification of the sodium-liquid ammonia reduction reaction mixture a precipitate was formed. This precipitate was identified on the basis of its spectrum, hydrolysis to phenylpyruvic acid, and analysis as tetrahydro-2-(4-hydroxybenzyl)-5-methyl-4-oxoglyoxaline-2-carboxylic acid, IX.

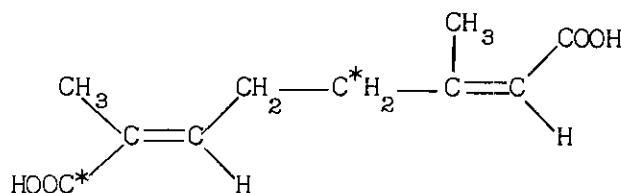


IX

This compound apparently arises from ring opening followed by reclosure of the benzaldiketopiperazine.

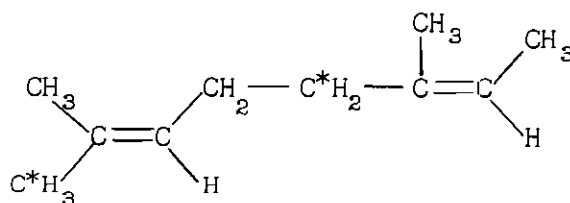
Birch continued his work on the biosynthesis of the terpenoid portion of mycelianamide (9). In 1962 he reported the following work on the stereochemistry and labeling of the methylgeraniolene obtained from mycelianamide which had been isolated from a culture grown with [2-C<sup>14</sup>]-mevalonic acid. The methylgeraniolene, obtained by sodium-liquid ammonia reduction of mycelianamide, was biochemically oxidized by feeding it to a rabbit into Hilderbrandt's acid, X. The stereochemistry of this compound was determined by comparison of the N.M.R.

spectrum of the dimethyl ester with the spectra of known related compounds. The labeling pattern was determined by careful degradation.



X

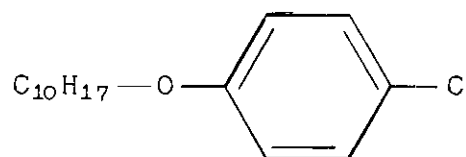
If it is assumed there was no isomerization or equilibration of the double bonds, the stereochemistry and labeling pattern of the methylgeraniolene would be shown by Formula XI.



XI

Summarizing the pertinent data which led to the assignment of Formula VI for mycelianamide, we should consider the following observations. Mycelianamide contains a terpenoid portion, which is attached to the molecule by a phenoxide bond, as evidenced by the isolation of p-myceloxybenzamide by the mild ammonia hydrolysis of mycelianamide. The identification of p-myceloxybenzamide by dilute acid hydrolysis to mycelene and p-hydroxybenzoic acid indicates that myceloxy function is

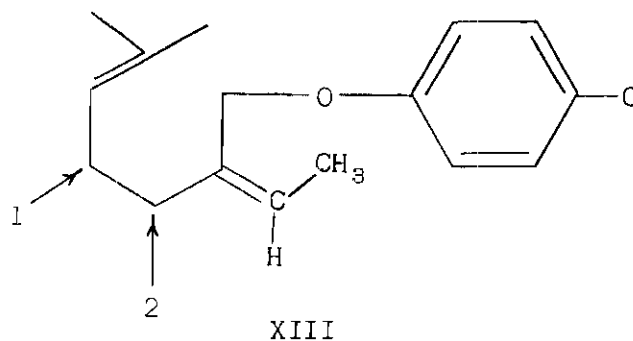
probably allylic. This hydrolysis also indicated that the terpene portion was attached to a benzene ring by an ether linkage. This benzene ring, as present in the intact molecule, also carries some other function in the para position which gives rise to the amide group observed in p-myceloxybenzamide. Therefore we can safely write the partial structure XII for mycelianamide.



XII

Insight as to the exact nature of the terpenoid portion was given by the fact that methylgeraniolene was obtained by the action of sodium and liquid ammonia on mycelianamide. This reagent would not be expected to have any profound effect on the carbon skeleton of the methylgeraniolene. The fact that the terpene portion was reductively cleaved by this reagent was a further indication of an allylic phenoxide moiety within the molecule. Having thus ascertained the carbon skeleton of the terpene, there remained the problem of the position of attachment of the methylgeraniolene to the phenol. Mycelianol was shown not to be identical with geraneol. p-Myceloxxybenzamide differs slightly from synthetic p-geranyloxybenzamide. Although levulaldehyde should be an ozonolysis product if a geranyl group were present, ozonolysis of p-myceloxxybenzamide produced only acetone and acetaldehyde.

Therefore, the partial structure, XIII, can be proposed.



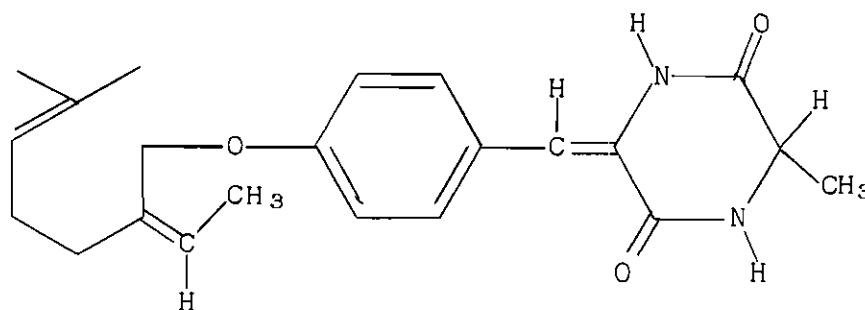
Attachment of the ether linkage at positions 1 and 2 evidently was ruled out on the basis of the C-methyl determination and the very distinct similarity of p-geranyloxy and p-myceloxy benzoic acids and amides. Biogenetic considerations also favor the structure shown.

The assignment of the configuration of the ethylidene double bond was made on the basis of N.M.R. studies done on Hilderbrandt's acid derived from methylgeraniolene.

Thus, seventeen of the carbon atoms, twenty-one of the hydrogen atoms and one oxygen atom have been accounted for by this partial structure XIII.

In determining the remainder of the structure, it must be first recognized that mycelianamide is a bis-hydroxamic acid. The data which supported this postulation were: the weakly acidic nature of mycelianamide, the formation of a ferric complex, and the presence of two oxygen atoms which are easily lost by reduction. The reduction of mycelianamide by zinc and acetic acid resulted in the loss of two

oxygen atoms. The resulting compound, deoxymycelianamide, is neither acidic nor gives a ferric test. The ultraviolet chromophore of mycelianamide was not greatly disturbed by this reduction. Deoxymycelianamide showed infrared absorption at  $5.95\ \mu$ , characteristic of cyclic, unstrained amides. Acid hydrolysis of deoxymycelianamide gave p-hydroxyphenylpyruvic acid and alanine, in addition, one presumes, to ammonia and the terpene portion of the molecule. These fragments account for all of the atoms of deoxymycelianamide, appropriate allowance having been made for the hydrolysis. Therefore, Formula XIV for deoxymycelianamide was consistent with the data.



XIV

The simple matter of replacement of the amidic protons with hydroxyl groups, which were removed by reduction, results in an acceptable structure for mycelianamide.

Some observations are not readily explained. The reasons for these observations may lie in the rather obscure hydroxylamine chemistry. For instance, the products observed upon hydrolysis of mycelianamide.

Acetaldehyde and alanine were observed when one would anticipate  $\alpha$ -hydroxylamino propionic acid. The mechanism of the hydrolysis of the 1,4-dihydroxy-2,5-diketopiperazine portion of the molecule is not apparent. Similarly the products of ozonolysis of mycelianamide are anomalous.

In 1961 it was decided to undertake the synthesis of mycelianamide and some related compounds. The purpose of this work was to obtain synthetic proof of the structure VI. In view of the bacteriostatic properties it was also felt that the establishment of a synthetic route to the mycelianamide skeleton and its analogs might lead to a useful antibiotic substance.

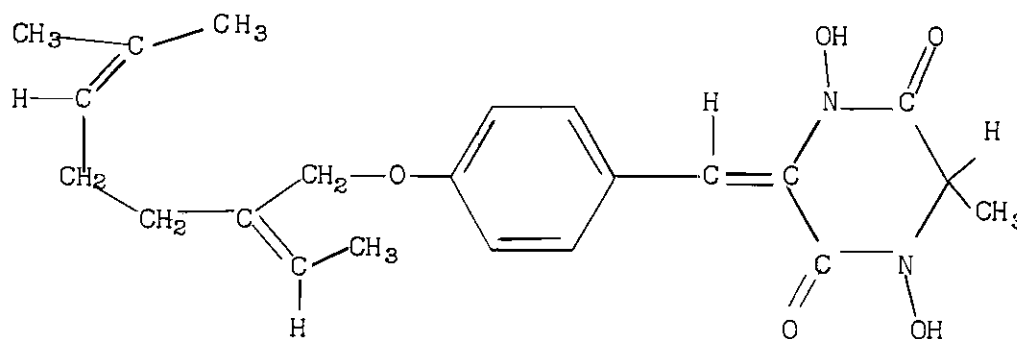
All hydroxamic acid derivatives isolated from nature show antibiotic properties. A cursory literature survey indicated that there were no general routes to N-substituted hydroxamic acid. It was therefore hoped that such a general method could be developed in the course of this research.



## CHAPTER II

### DISCUSSION OF RESULTS

The original aim of this research was the synthesis of myceli-  
anamide (VI).



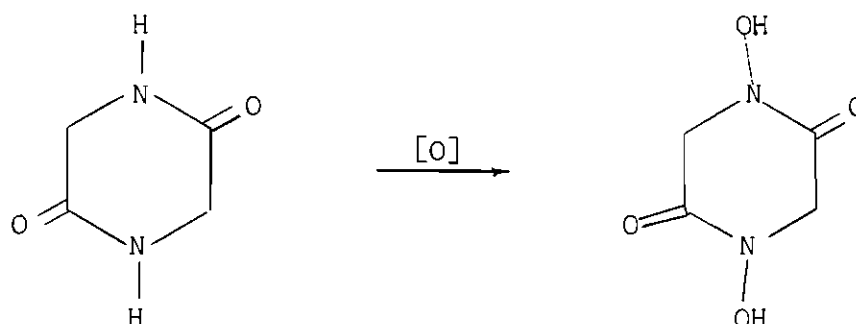
VI

This synthetic problem can be resolved into three parts: the synthesis of terpenoid, the aromatic, and the heterocyclic portions. The aromatic portion can be recognized as a residue of p-hydroxybenzaldehyde and therefore would not offer any synthetic problems. The terpenoid portion is related to many natural, available terpenes, but does not appear to be easily prepared by synthetic manipulations of these compounds. Therefore, it was necessary to prepare this portion by a total synthesis. The heterocyclic portion of the molecule presented a rather formidable problem in that there are no established methods of preparation of substituted hydroxamic acids of this type.

There are, of course, methods of preparing the simple 2,5-diketopiperazines. Therefore, it was decided that the preferable

approach would be to attempt to prepare 1,4-dihydroxy-2,5-diketopiperazine as a model compound and, if successful, to apply this method to the synthesis of the heterocyclic portion of mycelianamide. The attempted preparations of 1,4-dihydroxy-2,5-diketopiperazine can be divided into three categories. Direct oxidation of 2,5-diketopiperazine was tried. Ring closure or direct synthesis and cyclization of  $\alpha$ -hydroxylamino esters, both of which are analogous to established methods of preparation of 2,5-diketopiperazine (21), were also tried.

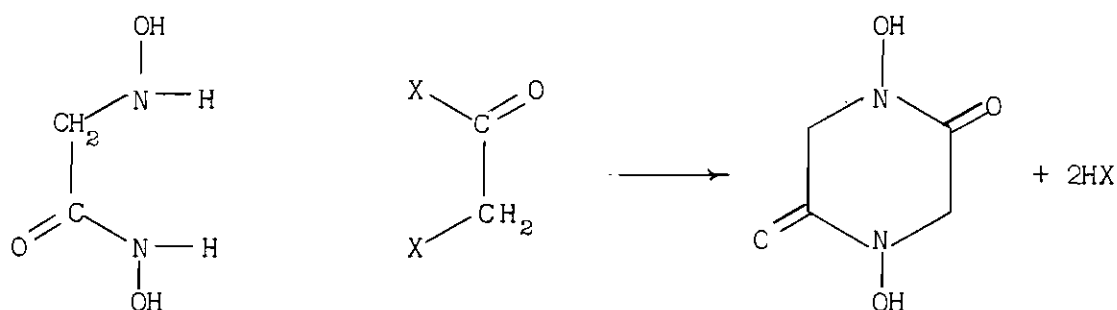
The direct oxidation of a diketopiperazine would involve the oxidative replacement of the amidic proton by an hydroxyl group:



There appears to be precedence for the oxidation of amides to hydroxamic acids, as (24), although no reference was found for work done with N-substituted amides. These experiments were generally carried out on a small scale. The reactants were mixed under various conditions and the success or failure of the experiment was determined by testing the product, after interfering substances had been removed, with ethanolic ferric chloride. A characteristic red-brown color indicates the presence of hydroxamic acids. The attempted oxidizing reactions listed in

the experimental portion of this work is by no means complete; it is, however, representative. Many of the reactions were carried out as little more than spot tests. Of all of the reactions of this nature which were tried, only the oxidations with permanganate and with peracetic acid showed any promise. In both cases, however, no pure compound was isolated.

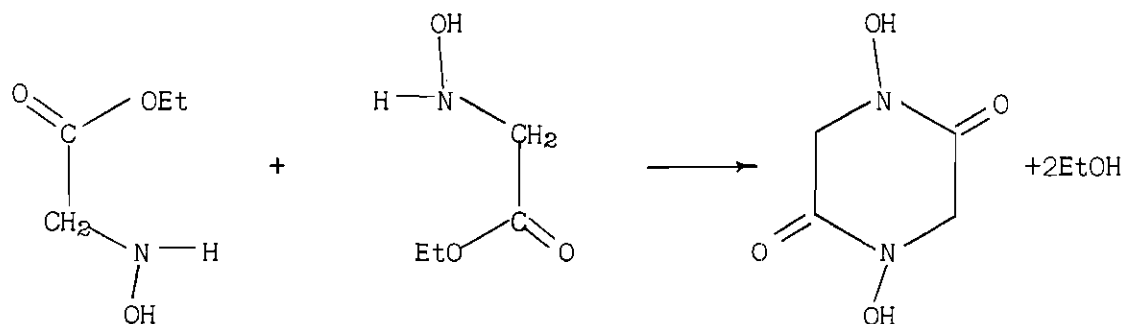
The ring closure methods attempted were based on the assumption that  $\alpha$ -hydroxylaminohydroxamic acids could be cyclized by  $\alpha$ -halo acid halides to give 1,4-dihydroxy-2,5-diketopiperazines:



The preparation of pure  $\alpha$ -hydroxylaminohydroxamic acids was unsuccessful primarily because the desired material, in the reaction mixture, is contaminated with a number of compounds with similar physical and chemical properties. They also appear to be relatively unstable compounds. This rendered purification quite difficult. The treatment of an enriched fraction of hydroxamic acids, obtained by ion exchange resin techniques, with  $\alpha$ -halo acid halides led to an inseparable mixture.

The next approach to the preparation of 1,4-dihydroxy-2,5-diketopiperazine was the reduction of  $\alpha$ -nitro esters to  $\alpha$ -hydroxylamino esters.

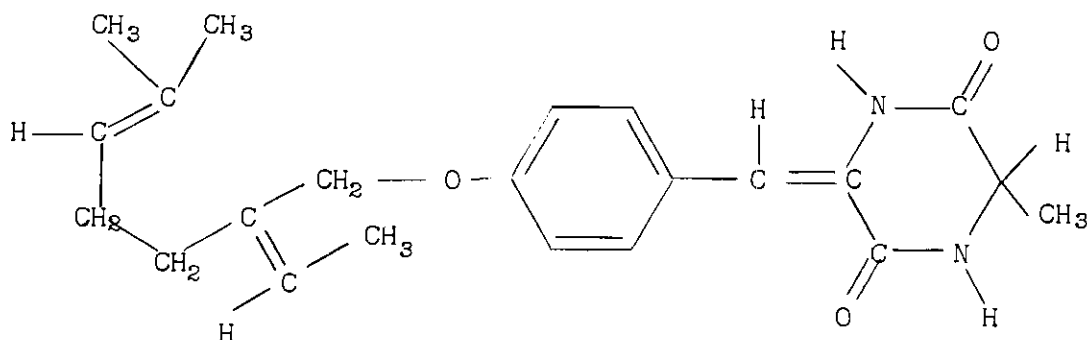
These compounds, it was hoped, would cyclize in a manner analogous to glycine ethyl ester:



An aqueous solution of glycine ethyl ester upon standing at room temperature forms 2,5-diketopiperazine. The catalytic hydrogenations appeared to proceed successfully in that the reaction mixture readily reduced silver ion to silver as would be expected of the hydroxylamino group. Attempts to isolate the hydroxylamino compound resulted in the isolation of the corresponding amino compound. The fact that the reduction reaction mixtures were noted to darken over a short period of time indicated that additional reactions took place after the reduction had been stopped. Attempts to induce cyclization of the  $\alpha$ -hydroxylamino esters by standing, heating, and base catalysis were unsuccessful. The chemical reduction of  $\alpha$ -nitro esters was also attempted (20). The  $\alpha$ -nitro ester was stirred in aqueous ethanolic solution with ammonium chloride and an equivalent amount of zinc dust. The inorganic products of the reaction were removed to yield a syrup. No pure compound or derivative could be isolated from this syrup.

Because of the apparent difficulty in constructing the hydroxamic acid portion of mycelianamide, it was decided to change the aim

of the research to the synthesis of deoxymycelianimide, XV.

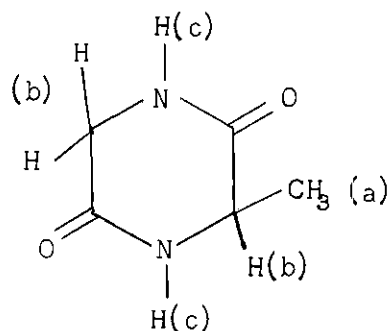


XV

The conversion of mycelianamide to deoxymycelianamide takes place under rather mild conditions. Thus it was felt that no structural rearrangements had taken place and that a synthesis of deoxymycelianamide would be very strong evidence for the correct structure of mycelianamide.

3-Methyl-2,5-diketopiperazine, the heterocyclic portion of deoxymycelianamide, was first prepared by heating a mixture of glycine and alanine ethyl esters without solvent at 100°. The yield of this reaction was quite low because of the difficulty of separating the desired product from the by-products. Chloroacetyl alanine ethyl ester was prepared by acylation of alanine ethyl ester with chloroacetyl chloride in a two-phase reaction. Low temperature was found to favor the formation of the desired product, probably by retarding hydrolysis of the acyl halide. The resulting chloroacetyl alanine ethyl ester was cyclized by heating with methanolic ammonia. This is thought to proceed by ammonolysis of the ester followed by displacement of the halide by the amide (21). The reaction can be visualized as proceeding with

equal facility by displacement of the halide by ammonia followed by ring closure. The product as obtained from the reaction mixture, in about 80 per cent yield, is quite pure. The N.M.R. spectrum of 3-methyl-2,5-diketopiperazine in trifluoroacetic acid solution was recorded and the assignments of the absorptions are shown in Formula XVI.



XVI

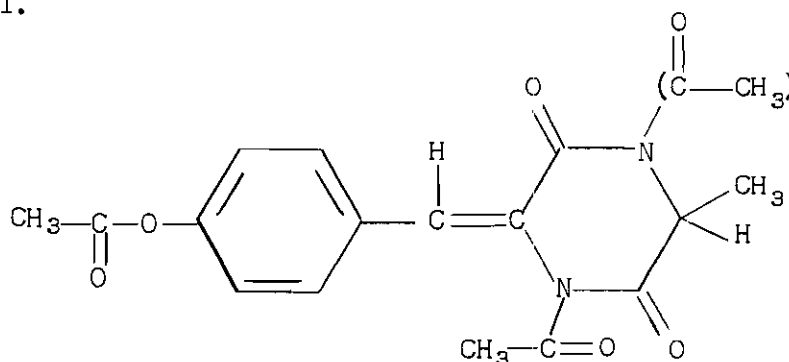
- (a) 8.30  $\tau$  (3H, doublet,  $J = 7$  cps.)
- (b) 5.52  $\tau$  (3H, complex multiplet)
- (c) 1.66  $\tau$  (2H, poorly resolved doublet)

p-Acetoxybenzaldehyde was prepared by acetylation of commercial p-hydroxybenzaldehyde using acetic anhydride and a trace of pyridine. 3,6-bis-(p-Acetoxybenzylidene)-2,5-diketopiperazine was prepared in 56 per cent yield as a model compound using the method of Richardson, Welch, and Calvert (14).

3-Methyl-2,5-diketopiperazine was coupled with p-acetoxybenzaldehyde by boiling it in the presence of acetic anhydride and anhydrous sodium acetate. The product, isolated in 26 per cent yield, appeared on the basis of elemental analysis to be a triacetyl derivative. The

infrared spectrum showed absorptions which can be assigned to two types of carbonyl groups, 5.63 and 5.85  $\mu$ .

The N.M.R. spectrum of this material was consistent with its formulation as a mixture of the di- and tri-acetyl compound, as shown in Formula XVII.



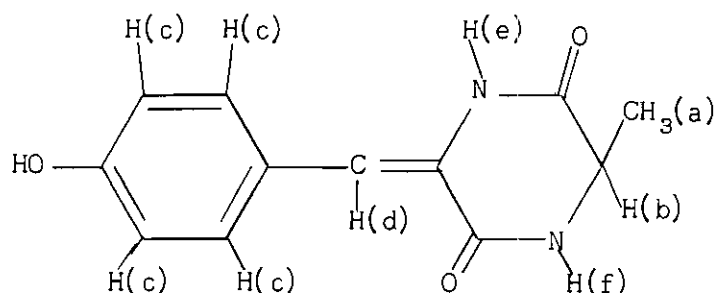
XVII

It has been reported that the reaction of an aromatic aldehyde with a diketopiperazine containing only one methylene group in the presence of acetic acid and sodium acetate leads to a product in which one of the nitrogen atoms is acetylated (22).

The ultraviolet spectrum of this material,  $\lambda_{\text{max}}$  at 226 and 317  $m\mu$ , was similar to that of deoxymycelianamide,  $\lambda_{\text{max}}$  at 225 and 317  $m\mu$ , as would be expected since the chromophore of the two compounds is very nearly the same.

The acetyl groups of XVII were easily removed by the action of aqueous-ethanolic ammonia, yielding 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine, XVIII. This compound was identified on the basis of its elemental analysis and spectral data. The infrared spectrum

of this compound showed absorptions at 3.3, 5.68, 5.90, 6.14, and 6.26  $\mu$  and is thought to be consistent with the assigned structure XVIII. The ultraviolet spectrum,  $\lambda_{\max}$  at 224  $m\mu$  ( $\epsilon = 12,100$ ) and 317  $m\mu$  ( $\epsilon = 18,700$ ), is quite similar to the values reported for deoxymycelianamide,  $\lambda_{\max}$  at 225  $m\mu$  ( $\epsilon = 14,000$ ), and 317  $m\mu$  ( $\epsilon = 2,200$ ) (6). The  $\tau$  values assigned to the N.M.R. absorptions are shown below Formula XVIII. These assignments were made on the usual basis of peak position and splitting.

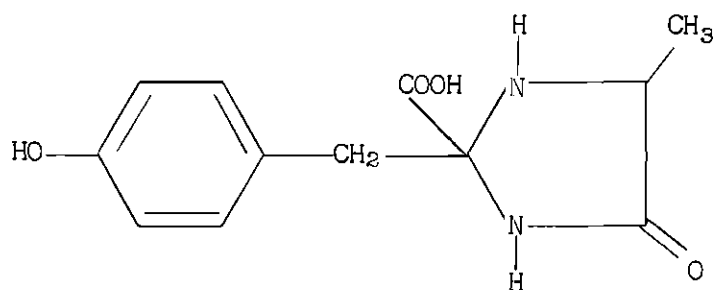


XVIII

- (a) 8.35  $\tau$  (3H, doublet,  $J = 7$  cps.)
- (b) 5.52  $\tau$  (1H, poorly resolved multiplet)
- (c) 3.00, 2.68  $\tau$  (doublets,  $J = 10$  cps.,  $A_2B_2$  system)
- (d) 2.80  $\tau$  (singlet)
- (e) } 1.59, 0.80  $\tau$  (broad singlets)
- (f) }

It was thought that this compound, XVIII, was an intermediate in the transformation reported by Birch (8) of mycelianamide to tetrahydro-2-(4-hydroxybenzyl)-5-methyl-4-oxoglyoxaline-2-carboxylic acid XIX, by the action of sodium in liquid ammonia followed by water. In



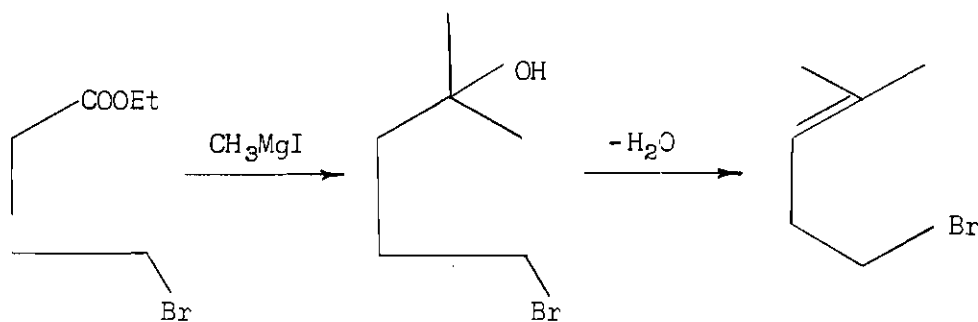


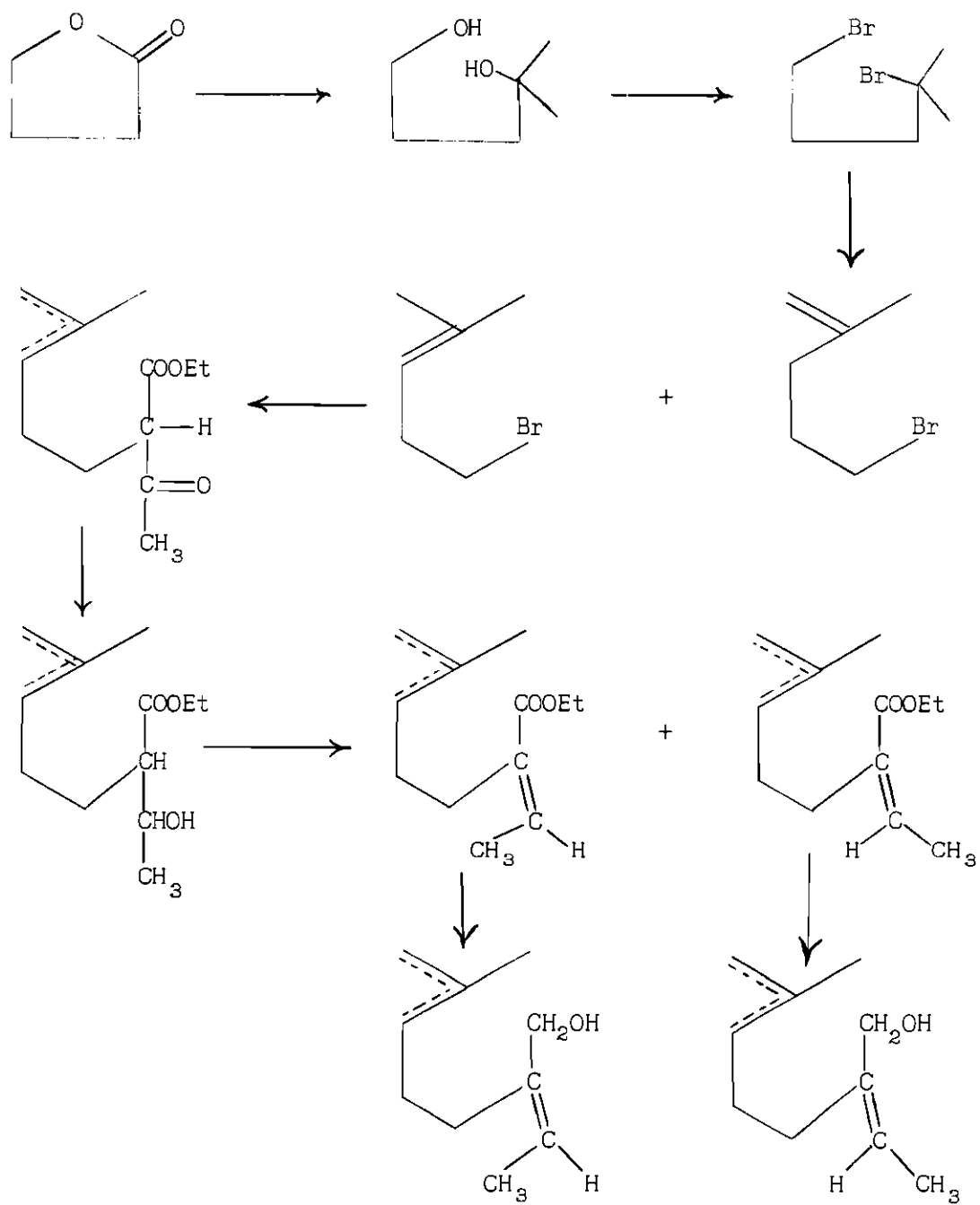
XIX

order to test this hypothesis, samples of XVIII were treated with sodium in liquid ammonia, sodium amide in liquid ammonia, and aqueous sodium hydroxide in hope of isolating XIX. No XIX, however, was found.

Having achieved the synthesis and coupling of the aromatic and heterocyclic portion of deoxymycelianamide the next step was to undertake the synthesis of the terpenoid portion of the molecule. An outline of the method employed is shown on the following page.

The original synthetic route to 4-methyl-3-pentenyl bromide involved the preparation of ethyl  $\gamma$ -bromobutyrate (10). It was hoped that treatment of this compound with two moles of methyl magnesium iodide would yield 4-methyl-4-hydroxypentyl bromide. This compound could then be dehydrated to the desired 4-methyl-3-pentenyl bromide.

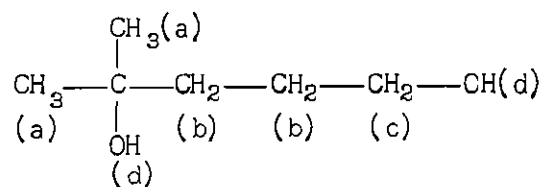




## Outline of the Synthesis of the Terpenoid Portion of Mycelianamide

It appeared that the reaction with methyl magnesium halide did not proceed in the normal fashion; the products of the reaction were not identified.

The synthetic route utilized to prepare 4-methyl-3-pentenyl bromide was essentially that of Ruzicka and Liguori (11). They, however, prepared the 1,4-dihydroxy-4-methyl pentane by a longer and more difficult route. The addition of two moles of methyl magnesium halide to butyrolactone proceeded without difficulty. The product, obtained in 82 per cent yield, was identified on the basis of the boiling point reported by Ruzicka and Liguori (11) and spectral data. The infrared absorption at  $2.95 \mu$  was assigned to the hydroxyl groups. The lack of absorption in the carbonyl region indicated the product was not contaminated with butyrolactone. The assignments of the absorptions of the N.M.R. spectrum are shown in Formula XX.

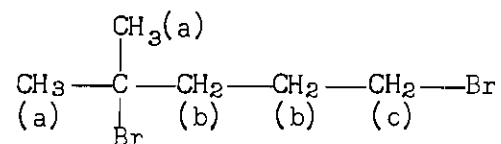


XX

- (a) 8.78  $\tau$  (6H, singlet)
- (b) 8.45  $\tau$  (4H, poorly resolved multiplet)
- (c) 6.40  $\tau$  (2H, triplet,  $J = 9$  cps.)
- (d) 5.62  $\tau$  (2H, singlet)

The conversion of this diol to the dibromide proceeded without difficulty by treatment with hydrogen bromide. The product, obtained

in 85 per cent yield, was identified on the basis of its boiling point and spectral data. The assignments of the N.M.R. absorptions observed are shown in Formula XXI.



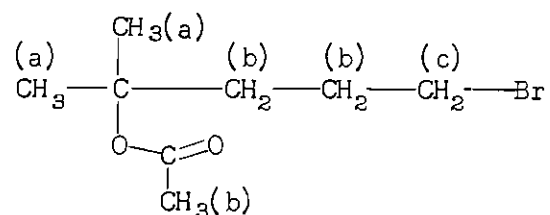
XXI

- (a) 8.27  $\tau$  (6H, singlet)
- (b) 8.00  $\tau$  (4H, complex multiplet)
- (c) 6.57  $\tau$  (2H, triplet,  $J = 6$  cps.)

The selective dehydrohalogenation of 1,4-dibromo-4-methylpentane to 4-methyl-3-pentenyl bromide did not proceed well. The reaction of the dibromide with alcoholic potassium hydroxide at room temperature appeared to give none of the desired product; no starting material was recovered. Dehydrohalogenation using Ruzicka and Liguori's method (11) of potassium benzoate in acetic acid was found to be quite sensitive to the presence of water. This difficulty was overcome by using acetic anhydride in the solvent to remove any water present.

The method also afforded a by-product which was isolated and identified, on spectral grounds only, as 1-bromo-4-methyl-4-acetoxypentane. The infrared absorption observed at  $5.73 \mu$  indicated the presence of an ester group. The N.M.R. absorption at  $6.58 \tau$  indicated the presence of a methylene group of a primary bromide, rather than in

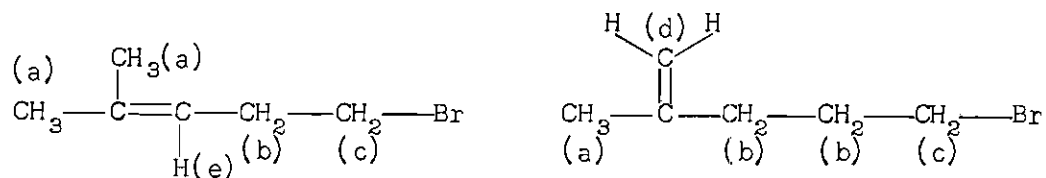
the vicinity of 6.0  $\tau$  which would indicate a methylene group of a primary acetate. The assignments of the observed N.M.R. absorptions are shown in Formula XXII.



XXII

- (a) 8.59  $\tau$  (6H, singlet)
- (b) 8.10  $\tau$  (7H, poorly resolved multiplet)
- (c) 6.58  $\tau$  (2H, triplet,  $J = 6$  cps.)

Ruzicka and Liguori (11) report the product of their method of dehydrohalogenation to be pure 1-bromo-4-methyl-3-pentene. This was not found to be the case when the product of the reaction was examined by N.M.R. spectrometry. Two isomeric products were found to be present. The assignments of the N.M.R. absorptions observed for this mixture are shown in Formula XXIII.



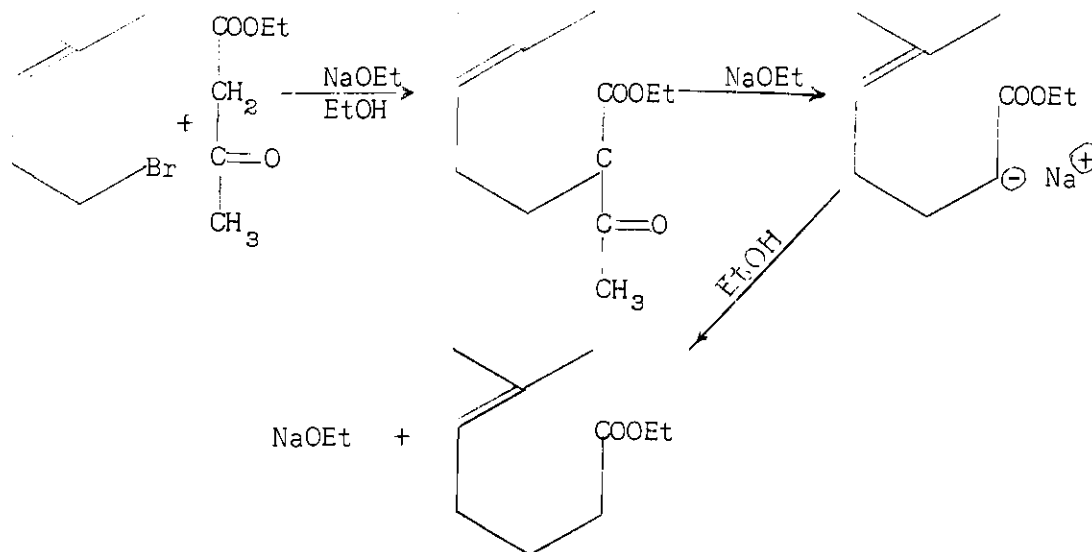
XXIII

- (a) ca. 8.3  $\tau$  (2 peaks)
- (b) 7.0-8.2  $\tau$  (several peaks)
- (c) 6.5 - 7  $\tau$  (several peaks)
- (d) 5.30  $\tau$  (2.2 integration units, singlet)
- (e) 4.89  $\tau$  (3 integration units, triplet,  $J = 7$  cps.)

Integration of the (d) and (e) absorptions led to the conclusion that the trisubstituted olefin was present to the extent of 83 per cent and the vinylidene olefin, 17 per cent. Vapor phase chromatography of the mixture showed that two compounds, of very nearly the same retention time, were present in the ratio of about 80 : 20.

The product of the reaction was found to boil over a very narrow range; thus separation by distillation was impractical. The two compounds were not resolved by preparative scale vapor phase chromatography. Therefore it was decided to utilize the mixture obtained for the remainder of the synthetic scheme. It should be remembered that the vinylidene isomer was never removed or completely isomerized to the trisubstituted olefin during the remainder of the synthetic sequence. Any reference to that portion of a molecule arising from this residue implies a mixture of the two double bond isomers.

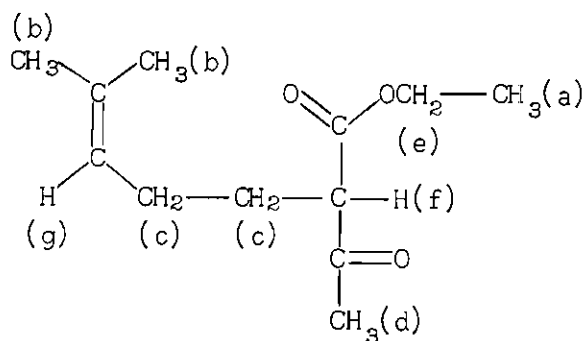
The next step in the synthesis of the terpenoid portion of deoxymycelianamide was the alkylation of ethyl acetoacetate with 1-bromo-4-methyl-3-pentene. The procedures employed for this reaction were adapted from Organic Synthesis (12). The only difficulty encountered was the isolation of ethyl 6-methyl-5-heptenoate, rather than the desired product. This compound undoubtedly arose by the sequence shown on the following page. The ethyl 6-methyl-5-heptenoate was identified on the basis of its elemental analysis and spectral data. The infrared spectrum showed only one absorption in the carbonyl region at 5.76  $\mu$ . This indicated that the compound was not the desired  $\beta$ -keto



ester. The N.M.R. spectrum did not show any absorptions which could be assigned to an acetyl group. It did indicate the presence of a carbethoxy group at 8.82  $\tau$  (triplet) and 5.96  $\tau$  (quartet), methylene groups and olefinic methyl groups 7.6-8.5  $\tau$  (several peaks), terminal vinylidene group, 5.38  $\tau$  (singlet), and trisubstituted olefin 4.90  $\tau$  (triplet,  $J = 7$  cps.) Integration of the N.M.R. spectrum indicated 83 per cent isopropylidene and 17 per cent vinylidene isomers were present.

When the alkylation was carried out using a shorter reaction time and excess 1-bromo-4-methyl-3-pentene, the desired ethyl 2-acetyl-6-methyl-5-heptenoate was obtained in 52 per cent yield, based on the alkylating bromide, or 75 per cent based on the ethyl acetoacetate. The compound was identified on the basis of its elemental analysis and spectral data. The infrared spectrum showed absorptions at 5.72 and

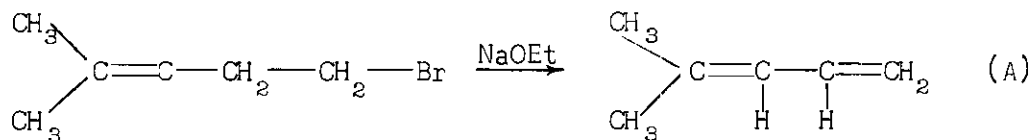
5.81  $\mu$ , indicating the presence of both ketone and ester groups. The assignments of the absorptions observed in the N.M.R. spectrum are shown in Formula XXIV.



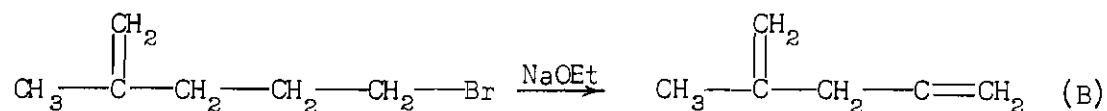
XXIV

- (a) 8.78  $\tau$  (triplet,  $J = 8$  cps.)
- (b) 8.2-8.5  $\tau$  (several peaks)
- (c) 7.9-8.2  $\tau$  (several peaks)
- (d) 7.85  $\tau$  (singlet)
- (e) 5.83  $\tau$  (quartet,  $J = 7$  cps.)
- (f) 6.53  $\tau$  (poorly resolved triplet)
- (g) 4.85  $\tau$  (poorly resolved triplet)

An absorption was also noted at 5.28  $\tau$  which was attributed to the terminal vinylidene isomer which was mentioned earlier. The integration of the absorptions at 4.85 and 5.28  $\tau$  indicated that the two isomers were present in nearly equal proportions. This increase in the proportion of the vinylidene isomer can be accounted for by a selective dehydrohalogenation side reaction which must have taken place.

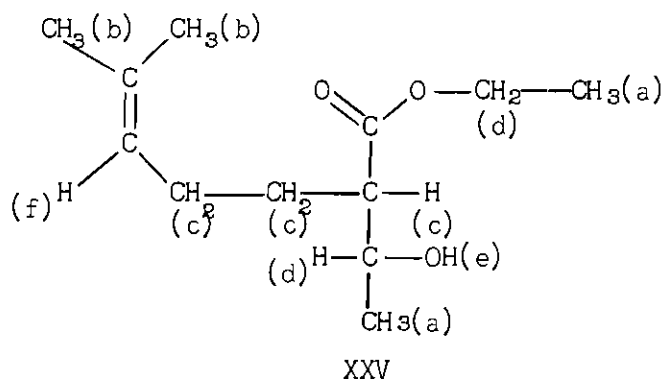






Reaction A probably takes place with greater facility than reaction B, as A leads to a conjugated olefin. This would reduce the availability of the trisubstituted olefinic bromide for alkylation of ethyl acetoacetate.

The next step in the synthesis involved the reduction of the ketone group of ethyl 2-acetyl-6-methyl-5-heptenoate to the corresponding alcohol. The reaction proceeded without difficulty by dissolving the ketone in ethanol and adding an excess of powdered solid sodium borohydride. The yield of crude product was 95 per cent of theory. Possible difficulties which were anticipated, but not observed, were reverse Claisen, aldol condensation or hydrolysis of the ester function. All of these reactions could have taken place in the basic aqueous solution. The product was identified by its elemental analysis and spectral data. The infrared spectrum of the product differed from the reactant by the disappearance of the absorption assigned to the ketone group ( $5.81 \mu$ ) and the appearance of an hydroxyl group ( $2.90 \mu$ ). The assignment of the N.M.R. absorptions was difficult because of overlapping absorptions. Absorption was noted at  $5.10 \tau$  and is attributed to the terminal vinylidene isomer. Integration of the  $5.10$  and  $4.65 \tau$  peaks indicated the vinylidene and trisubstituted olefin were present in a ratio of 56 : 44, respectively.

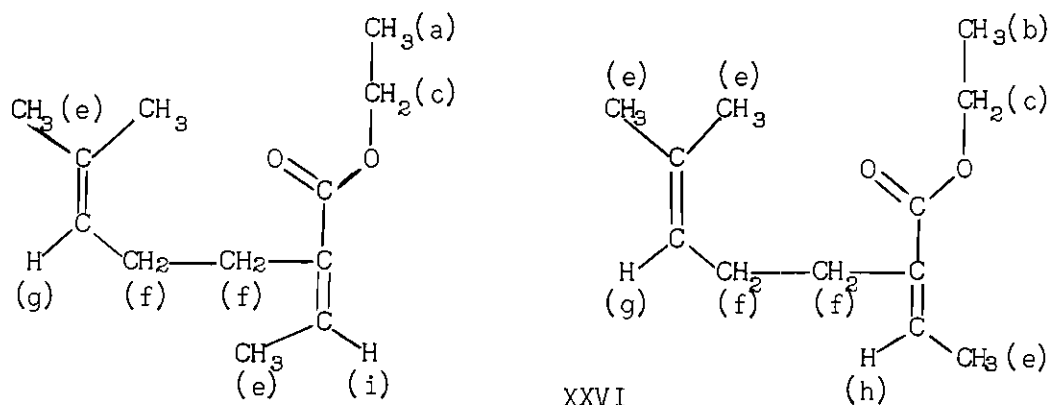


- |   |   |
|---|---|
| (a) <u>ca.</u> 8.7 $\tau$ (triplet, $J = 8$ cps.) | (d) 5.60 $\tau$ (quartet, $J = 7$ cps.)   |
| (b) <u>ca.</u> 8.3 $\tau$ (several peaks)         | (e) 6.11 $\tau$ (broad singlet)           |
| (c) 7.3-8.2 $\tau$ (several peaks)                | (f) 4.65 $\tau$ (poorly resolved triplet) |

The dehydration of this  $\beta$ -hydroxy ester to the corresponding  $\alpha,\beta$ -unsaturated ester, although it appeared to be quite simple, proved to be one of the most difficult steps of the synthesis. The methods which were not successful, treatment with iodine, phosphorus oxychloride, *p*-toluenesulfonic acid, and thionyl chloride were adaptations of methods recommended by Organic Reactions for the dehydration of products of the Reformatski reaction (23). These methods appear to be successful for  $\beta$ -hydroxyesters which are not substituted on the  $\alpha$ -carbon atom. They were not useful in this case. Iodine and *p*-toluenesulfonic acid did not effect reaction. Phosphorus oxychloride appeared to cause cyclization or drastic molecular rearrangement on the basis of the N.M.R. spectrum. The spectrum showed only very slight absorption in the region associated with olefinic protons, below 5.5  $\tau$ . Treatment with thionyl chloride and pyridine resulted in the isolation of the respective chloro compound. This compound was identified on spectral

evidence only. The infrared spectrum showed absorption at  $5.77\ \mu$ , assigned to the ester carbonyl group, but no absorption was observed in the region associated with hydroxyl groups  $2.5\text{--}3.1\ \mu$ . The N.M.R. spectrum of this compound was very similar to the spectrum of the starting material except that the absorption assigned to hydroxyl,  $6.11\ \tau$ , was absent.

The successful method involved the preparation of the p-toluene-sulfonate of the alcohol and treatment of this compound with sodium ethoxide in ethanol. This procedure resulted in the isolation of the desired  $\alpha,\beta$ -unsaturated ester in 69 per cent yield. The compound was identified as a mixture of equal amounts of cis- and trans-ethyl 2-ethylidene-6-methyl-5-heptenoate on the basis of the elemental analysis and spectral data. The infrared spectrum showed the presence of an  $\alpha,\beta$ -unsaturated ester,  $\lambda_{\text{max}}$  at  $5.83\ \mu$ . The N.M.R. assignments of the cis and trans isomers were made according to values quoted in the literature (9). It is fortunate that the absorptions of the two configurations of the ethylidene proton were separated by  $0.9\ \tau$  units. This effect, which undoubtedly arises from increased deshielding of the ethylidene proton by the carbethoxy group when it is located in the cis configuration to this group, facilitated the assay and separation of this mixture of isomers. It is interesting to note that the absorptions of the protons of the ethyl groups are slightly different for the two isomers. The assignments of the absorption observed are shown in Formula XXVI.



(a) 8.76 $\tau$ (triplet, J = 7 cps.)	(e),(f) 7.5 - 8.5 $\tau$ (about ten peaks)
(b) 8.78 $\tau$ (triplet, J = 7 cps.)	(g) 4.87 $\tau$ (poorly resolved triplet)
(c) 5.82 $\tau$ (quartet, J = 7 cps.)	(h) 4.02 $\tau$ (quartet, J = 7 cps.)
(d) 5.85 $\tau$ (quartet, J = 7 cps.)	(i) 3.14 $\tau$ (quartet, J = 7 cps.)

Absorption was also noted at 5.28  $\tau$  and was assigned to the terminal vinylidene isomer of the isopropylidene group. Integration of the (h) and (i) absorptions indicate that the cis- and trans- (methyl-carbethoxy) isomers were present in equal amounts. The relative proportions of the absorptions at 4.87 and 5.28  $\tau$  appeared to be unchanged.

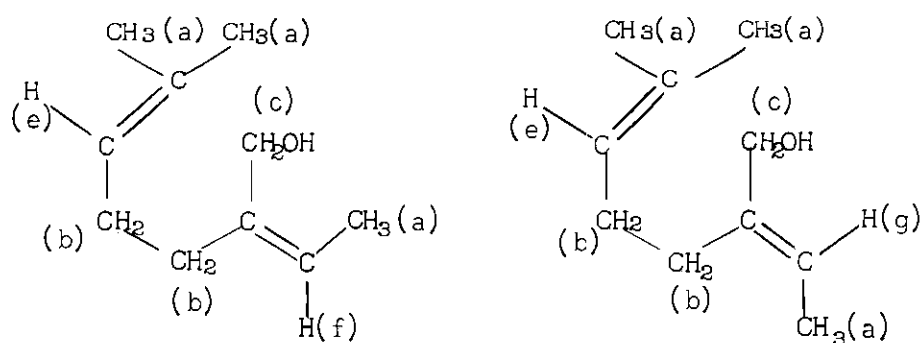
Since the dehydration reaction gave a mixture of cis and trans isomers [and only the cis- (methyl-carbethoxy) isomer was desired] several attempts were made to isomerize the trans ethylidene double bond to the cis. Methods tried included acid and base catalysis and ultraviolet irradiation. None was successful.

The cis and trans isomers were separated by alumina chromatography; the column was eluted with benzene. In spite of the fact that an adequate ratio of adsorbent to compound was used (21 lbs. : 44 g.) the separation was not complete. Only the first and last few fractions

of the compound eluted consisted of pure isomers. The separation was assayed by N.M.R. spectroscopy by observing the absorptions at 3.12 and 4.01  $\tau$ . No separation of the mixture of vinylidene and trisubstituted olefin mixture was noted by examination of the absorptions at 4.87  $\tau$  and 5.28  $\tau$ .

The next reaction of the synthetic scheme was the reduction of the ester function of the ethyl 2-ethylidene-6-methyl-5-heptenoate with lithium aluminum hydride. A possible side reaction was that the ethylidene double bond might be partially reduced by this reagent, thus creating a difficult separation problem. It was found that the ethylidene double bond was indeed reduced to a small extent and the product of this side reaction was the aldehyde, 2-ethyl-6-methyl-5-heptenal. The by-product aldehyde was easily separated from the desired alcohol, 2-ethylidene-6-methyl-5-hepten-1-ol, by chromatography on silicic acid. The aldehyde was identified by its infrared and N.M.R. spectra. The infrared spectrum showed the characteristic absorptions at 3.65 and 5.82  $\mu$ . The N.M.R. spectrum showed absorption at 1.25  $\tau$ , assigned to the aldehydic proton, which was split into a doublet ( $J = 2.3$  cps.). This would indicate that the adjacent carbon atom carries one proton, which would be the case if the double bond had been reduced. This aldehyde undoubtedly does not exist as such in the lithium aluminum hydride solution. It probably is present as the enolate anion arising from 1,4 addition of hydrogen. A brief survey of the literature failed to disclose precedent for this observation.

Reduction of ethyl 2-ethylidene-6-methyl-5-heptenoate with lithium aluminum hydride in which the carbethoxy and methyl groups are located in a cis configuration led to a material, referred to as cis-mycelianol, which showed N.M.R. absorption at 5.92  $\tau$ . Reduction of the trans isomer gave a material, trans-mycelianol, showing N.M.R. absorption at 6.04  $\tau$ . Reduction of a mixture of the two isomers gave a material which showed absorption at both positions. It was hoped that this mixture of isomeric alcohols could be separated by means of the calcium chloride complexes, analogous to the separation of geranol and nerol. No separation was observed. The two isomeric mycelianols appeared to be very similar in all respects except the N.M.R. absorption at 6.04 or 5.92  $\tau$ . Because of the small amount of material on hand analytical data were not obtained. The infrared spectra of the two alcohols were nearly superimposable and showed absorptions which would be expected for this type of compound. The assignments of the N.M.R. absorptions observed of the pure cis and trans isomers are shown in Formula XXVII.



XXVII

(a) 8.42 $\tau$ , 8.30 $\tau$ (two peaks)	(e) 4.90 $\tau$ (poorly resolved triplet)
(b) 8.2-7.7 $\tau$ (several peaks)	(f) 4.65 $\tau$ (quartet, J = 7 cps.)
(c) 5.92 $\tau$ (singlet)	(g) 4.64 $\tau$ (quartet, J = 7 cps.)
(d) 6.04 $\tau$ (singlet)	

Absorption was also noted at  $5.30 \mu$ , which was assigned to the vinylidene double bond isomer of the isopropylidene group.

Because of the similarity of the structure of the synthetic alcohols to geraneol it was decided to utilize this readily available compound as a model. Geranyl bromide was prepared by the method of Schmidt (13) except that the product was not purified by distillation. It was hoped that purification could be effected with a smaller loss of material by chromatography. This was not the case. Upon contact with an adsorbing agent such as alumina or silicic acid, the compound underwent reaction, large amounts of heat were evolved, and the adsorbant turned dark purple. Examination of the crude geranyl bromide by N.M.R. and infrared spectroscopy indicated that it was relatively pure. The infrared spectrum indicated the absence of hydroxyl groups,  $2.9 \mu$ . The N.M.R. spectrum was very similar to that of geraneol, except there was no absorption which could be assigned to a hydroxyl group. Therefore, the compound was used without further purification.

In order to ascertain whether dimethylsulfoxide and potassium carbonate was a satisfactory medium for coupling phenols with allylic bromides p-geranyloxybenzoic acid was prepared by alkylation of ethyl p-hydroxybenzoate with geranyl bromide and subsequent hydrolysis.

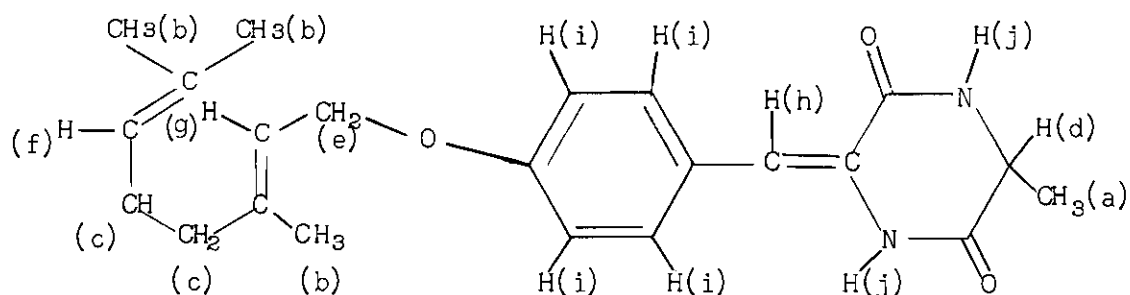
This reaction proceeded in 65 per cent yield to give p-geranyloxybenzoic acid, m.p.  $118^\circ$  [lit. m.p.  $118^\circ$  (5)]. Several attempts were made to alkylate the phenolic fragment of deoxymycelianamide, 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine, using geranyl bromide, potassium carbonate and dimethylsulfoxide. The alkylation yielded only

very small amounts of the desired product, the yield being only about five per cent of theory. When the method of Lauer and Labriola (17) was adopted, using anhydrous acetone as the solvent, the yield was increased to about 40 per cent.

That the reaction did not proceed well in dimethylsulfoxide may be explained by an experiment which indicated that geranyl bromide is completely destroyed by stirring for four hours with an equivalent amount of potassium carbonate in dimethylsulfoxide. It is felt that the use of acetone as solvent produced superior yields by reducing the reaction of geranyl bromide with basic species other than the phenoxide ion. It would seem reasonable that acetone would be a selective solvent, dissolving only the neutral phenol and the potassium salt of the phenol to any extent. Therefore, only these two species would be available for reaction with the geranyl bromide. Dimethylsulfoxide, on the other hand, dissolved the potassium carbonate and bicarbonate as well, making these bases available for reaction with the geranyl bromide. This hypothesis does not explain the reasonable yield obtained by alkylating ethyl p-hydroxybenzoate with geranyl bromide in dimethylsulfoxide solution.

The 3-(p-geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine, m.p. 172°, obtained was identified by elemental analysis and spectral data. The ultraviolet spectrum showed  $\lambda_{\text{max.}}$  at 226 m $\mu$  ( $\epsilon = 17,500$ ) and 317 m $\mu$  ( $\epsilon = 27,500$ ). The infrared spectrum (KBr) is reproduced as Figure 2. The N.M.R. spectrum is reproduced as Figure 1. The assignments of the N.M.R. absorptions are shown in Formula XXVIII.





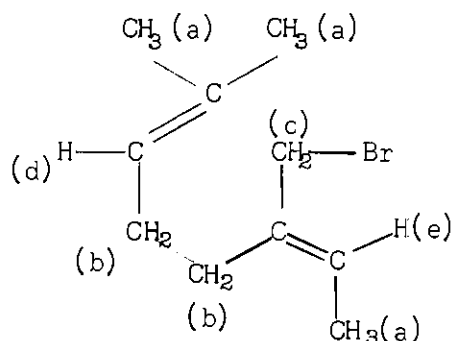
## XXVIII

- |   |   |
|---|---|
| (a) 8.76 $\tau$ (doublet, $J = 5$ cps.) | (f) 4.89 $\tau$ (poorly resolved triplet) |
| (b) 8.2-8.6 $\tau$ (several peaks)      | (g) 4.52 $\tau$ (poorly resolved triplet) |
| (c) 7.7-8.0 $\tau$ (about three peaks)  | (h) 3.11 $\tau$ (singlet)                 |
| (d) 5.71 $\tau$ (quartet, $J = 6$ cps.) | (i) 2.87 $\tau$ ( $A_2B_2$ system)        |
| (e) 5.42 $\tau$ (doublet, $J = 7$ cps.) | (j) 2.23, 188 $\tau$ (two broad singlets) |

Since the remainder of the reaction sequence appeared to proceed well using the model compound, geraneol, the methods used were applied to the synthetic alcohols, cis- and trans-2-ethylidene-6-methyl-5-hepten-1-ols (cis- and trans-mycelianols).

The pure trans-(methyl-hydroxymethyl) isomer, trans-mycelianol, was converted to the bromide using phosphorus tribromide and pyridine (13). The product, without purification, was examined using infrared and N.M.R. spectroscopy. The infrared spectrum indicated the absence of a hydroxyl group and the presence of double bonds ( $6.05 \mu$ ). The N.M.R. spectrum was recorded and the assignments of the absorptions observed are shown in Formula XXIX. Absorption at 5.28  $\tau$  attributed to the terminal vinylidene isomer was still present. Although an integration of the spectrum could not be obtained, it appeared by visual examination of the spectrum that the terminal vinylidene double bond

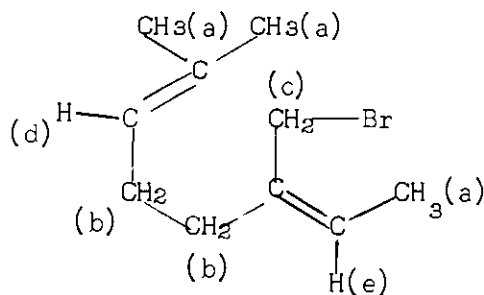
isomer, 5.28  $\tau$ , has been isomerized to a large extent into the tri-substituted olefin, 4.84  $\tau$ .



XXIX

- |                                    |   |
|------------------------------------|---|
| (a) 8.2-8.6 $\tau$ (several peaks) | (d) 4.84 $\tau$ (poorly resolved triplet) |
| (b) 7.6-8.0 $\tau$ (several peaks) | (e) 4.28 $\tau$ (quartet, $J = 7$ cps.)   |
| (c) 6.00 $\tau$ (singlet)          |   |

The pure cis-(methyl-hydroxymethyl) isomer, cis-mycelianol, was converted into the bromide by the same method. The infrared spectrum was virtually identical with that of the trans-bromide. The N.M.R. spectrum was recorded and showed the same isomerization of the terminal vinylidene olefin. The assignments of the N.M.R. absorptions are shown in Formula XXX.



XXX

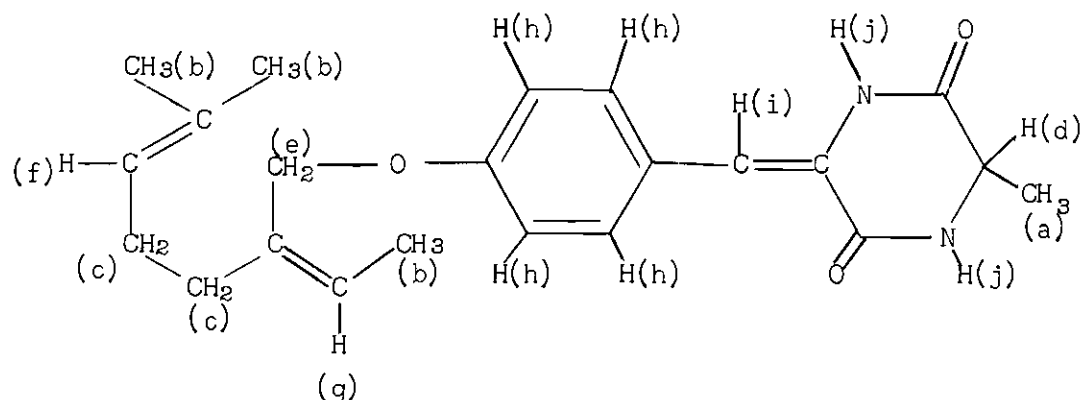
- |                                    |   |
|------------------------------------|---|
| (a) 8.2-8.6 $\tau$ (several peaks) | (d) 4.82 $\tau$ (poorly resolved triplet) |
| (b) 7.6-8.0 $\tau$ (several peaks) | (e) 4.45 $\tau$ (quartet, $J = 6$ cps.)   |
| (c) 5.98 $\tau$ (singlet)          |   |

It will be noted that the absorptions of the ethylidene proton are in different positions depending on the configuration of the alcohol reacted. This would indicate that there was no equilibration of configuration around this double bond during reaction.

It was noted that both of these bromides underwent reaction in the presence of alumina, analogous to the observed behavior of geranyl bromide.

The cis-(methyl-bromoethyl)-1-bromo-2-ethylidene-6-methyl-5-heptene, cis-mycetyl bromide, was used to alkylate 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine using acetone as solvent and potassium carbonate as base. The 3-[p-(2-ethylidene-6-methyl-5-heptenoxy)-benzylidene]-6-methyl-2,5-diketopiperazine was obtained in about 40 per cent yield and showed m.p. 160-161°. Good analytical data were not obtained for this compound. It is felt that a fraction of the terpenoid portion of the molecule was lost during the processing of the analytical sample, either by acid hydrolysis during recrystallization, as reported by Oxford (4), or by pyrolysis during drying, analogous to the behavior of ethyl p-geranyloxybenzoate reported by Lauer (17). The ultraviolet spectrum showed  $\lambda_{\text{max.}}$  at 226  $m\mu$  ( $\epsilon = 17,500$ ) and 317  $m\mu$  ( $\epsilon = 25,500$ ). The infrared spectrum (KBr) of the isolated compound is reproduced as Figure 5. The N.M.R. spectrum is reproduced as Figure 6. The assignments of the N.M.R. absorptions are shown in Formula XXXI.

Absorption was also noted at 5.26  $\tau$  which was assigned to the terminal vinylidene isomer of the isopropylidene group. A small

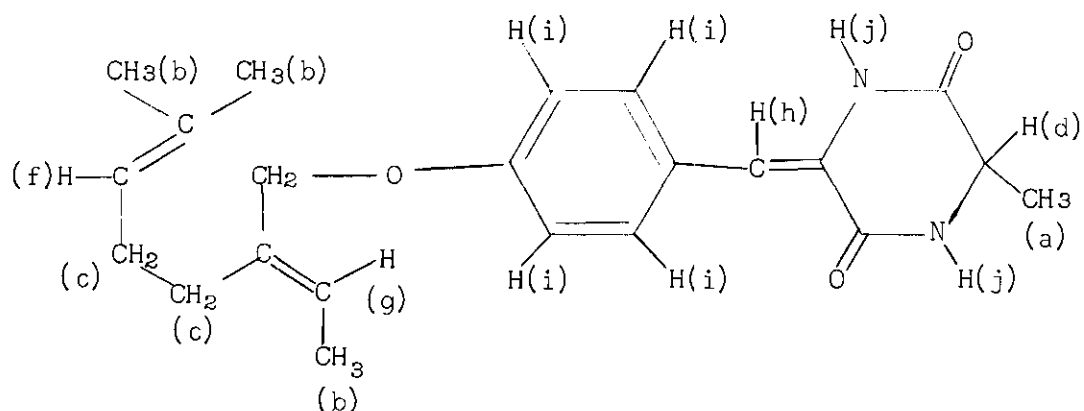


XXXI

- |   |  |
|---|--|
| (a) 8.82 $\tau$ (doublet, $J = 6$ cps.) | (f) 4.83 $\tau$ (poorly resolved triplet)  |
| (b) 8.1-8.6 $\tau$ (several peaks)      | (g) 4.35 $\tau$ (poorly resolved quartet)  |
| (c) 7.6-8.0 $\tau$ (several peaks)      | (h) 2.78 $\tau$ ( $A_2B_2$ system)         |
| (d) 5.62 $\tau$ (quartet, $J = 7$ cps.) | (i) 3.04 $\tau$ (singlet)                  |
| (e) 5.39 $\tau$ (singlet)               | (j) 2.01, 1.76 $\tau$ (two broad singlets) |

multiplet also appears at about 6.4  $\tau$ ; this is thought to be due to an unknown impurity even though a similar absorption appears in the spectrum of the product obtained by zinc-acetic acid reduction of mycelianamide.

The reaction of the trans-(methyl-bromomethyl) 1-bromo-2-ethylidene-6-methyl-5-heptene, trans-mycelyl bromide, with the phenol fragment, 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine, proceeded in the same fashion as did the cis-isomer to give about 40 per cent yield of a product melting at 169-170°. The same difficulty was encountered in obtaining good analytical data. The ultraviolet spectrum showed  $\lambda_{\max.}$  at 227  $m\mu$  ( $\epsilon = 17,500$ ) and 317  $m\mu$  ( $\epsilon = 31,200$ ). The infrared spectrum is reproduced as Figure 3. The N.M.R. spectrum is reproduced as Figure 4. The assignments of the N.M.R. absorptions are shown in Formula XXXII.



XXXII

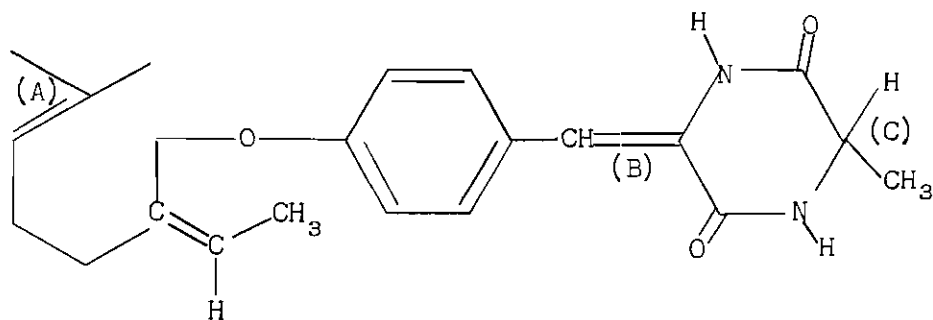
(a) 8.78 $\tau$ (doublet, $J = 6$ cps.)	(f) 4.82 $\tau$ (poorly resolved triplet)
(b) 8.1-8.7 $\tau$ (several peaks)	(g) 4.36 $\tau$ (poorly resolved triplet)
(c) 7.7-8.1 $\tau$ (poorly resolved multiplet)	(h) 3.10 $\tau$ (singlet)
(d) 5.71 $\tau$ (quartet, $J = 6$ cps.)	(i) 2.84 $\tau$ ( $A_2B_2$ system)
(e) 5.55 $\tau$ (singlet)	(j) 2.60, 2.00 $\tau$ (two broad singlets)

Absorption was also noted at 5.29  $\tau$ , which was attributed to the terminal vinylidene double bond isomer of the isopropylidene group.

The physical properties of an impure sample of mycelianamide obtained from Glaxo Laboratories were recorded. The melting point observed was 152-160°. The reported (6) value is 170-172° dec. The ultraviolet spectrum was recorded and showed  $\lambda_{\max.}$  at 232  $m\mu$  ( $\epsilon = 15,300$ ) and 320  $m\mu$  ( $\epsilon = 29,600$ ). This differs slightly from the reported values (6) of  $\lambda_{\max.}$  at 231  $m\mu$  ( $\epsilon = 11,500$ ) and 321  $m\mu$  ( $\epsilon = 23,000$ ). The infrared spectrum was recorded and is reproduced as Figure 8. The N.M.R. spectrum is shown as Figure 7. It will be noted that these spectra bear close resemblance to the respective spectra of the three synthetic compounds: cis-myceloxy-, trans-myceloxy-, and geranyloxy-benzylidene-methyldiketopiperazines.

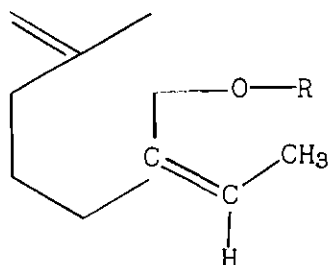
The entire lot of mycelianamide, 40 mg., was reduced with zinc and acetic acid, the method which Birch used to obtain deoxymycelianamide. This yielded a product, ca. 5 mg., which did not appear to be deoxymycelianamide. The material was noted to melt at 187°. This melting point was not affected by slow or rapid heating. Birch reported the melting point of deoxymycelianamide to be 170° (6). The ultraviolet spectrum showed  $\lambda_{\text{max}}$  at 227  $m\mu$  ( $\epsilon = 7,500$ ) and 317  $m\mu$  ( $\epsilon = 7,900$ ). The values reported by Birch were  $\lambda_{\text{max}}$  at 225  $m\mu$  ( $\epsilon = 14,000$ ) and 317  $m\mu$  ( $\epsilon = 2,200$ ). The infrared spectrum was recorded and is shown as Figure 9. The only infrared absorption reported by Birch is the carbonyl group at 5.96  $\mu$ . The N.M.R. spectrum is reproduced as Figure 10. These spectra show striking similarity to the respective spectra of the three synthetic materials. There are, however, differences.

The following conclusion from this work can safely be drawn: The synthesis of the compound, Formula XXXIII, which has been proposed as the structure for deoxymycelianamide by Birch (6), has been achieved. The following reservations, however, must be made. Structural feature (A): The material obtained by synthesis is a mixture of the isomer



XXXIII

shown in Formula XXXIII and also the terminal vinylidene isomer:



Structural feature (B): The stereochemistry around the benzylidene double bond is unknown, both in the synthetic and natural materials. Variable amounts of the cis and trans isomers could account for the rather variable ultraviolet extinction coefficients observed.

Structural feature (C): The deoxymycelianamide obtained by Birch showed optical activity,  $[\alpha]_D = -5^\circ$ . The material as synthesized is racemic.

Similarly it may be concluded that the respective trans-myceloxy compound has been synthesized. The same reservations concerning the structural features apply. The synthesis of the model compound, in which the terpenoid portion of the molecule is derived from geraneol can be considered successful with reservations (B) and (C).

The unavailability of authentic deoxymycelianamide for detailed comparison with compound XXXIII renders it impossible to draw any definite conclusion concerning their identity or nonidentity. Efforts to obtain an authentic sample of deoxymycelianamide or a sufficient amount of pure mycelianamide for the preparation of an authentic sample were unsuccessful.

## CHAPTER III

### EXPERIMENTAL

#### Instrumentation

The melting points reported are corrected and were determined using a K $\ddot{o}$ fler micro hot stage. The infrared spectra were recorded using either a Perkin-Elmer model 21 or model 137 recording spectrophotometer. Vapor phase chromatography was done using a Perkin-Elmer model 154 Vapor Fractometer. The ultraviolet spectra were determined in 95 per cent ethanol solution using a Beckman model DK recording spectrophotometer. The nuclear magnetic resonance spectra were determined using a Varian model A-60 spectrometer. All values quoted are on the  $\tau$  scale, relative to tetramethylsilane as an internal standard. The assignment of the N.M.R. absorptions was done on the usual basis of peak position and splitting. In most cases the assignments were confirmed by comparison with the spectra of known similar compounds. Microanalyses were performed by Galbraith Laboratories (Knoxville, Tennessee) and Huffman Laboratories (Wheatridge, Colorado).

#### Attempts to Prepare 1,4-Dihydroxy-2,5-diketopiperazine from 2,5-Diketopiperazine by Oxidation

A saturated aqueous solution of 2,5-diketopiperazine was prepared. An equal volume of ten per cent potassium persulfate was added and the solution divided into three parts. One part was heated on a



steam bath for two hours. The second part was permitted to stand at room temperature. The third portion was refrigerated for several days. When tested with ethanolic ferric chloride none of the reaction mixtures gave a positive ferric test.

Three portions of saturated aqueous solutions of 2,5-diketopiperazine were treated with an equal volume of persulfuric acid. One portion was refrigerated, the other two were permitted to stand at room temperature for one and two days, respectively. No positive ferric test was obtained when they were treated with ferric chloride solution.

Two samples of an aqueous solution of 2,5-diketopiperazine were placed in test tubes. One test tube contained 100 mg. of five per cent platinum on carbon, the other 100 mg. of five per cent palladium on carbon. Oxygen was bubbled through these test tubes for several days. Periodically, aliquots were tested with ferric chloride. No positive ferric tests were obtained.

Three small portions of a saturated aqueous solution of 2,5-diketopiperazine were treated with a few drops of 30 per cent hydrogen peroxide. One portion was heated on a steam bath, another was permitted to stand at room temperature, and the third was refrigerated. Treatment of aliquots of these solutions with ferric chloride solution failed to give a positive ferric test.

The above experiment was repeated with the exception that the aqueous solution contained a small amount of sodium tungstate. The results were the same.

A few milliliters of a saturated aqueous solution of 2,5-diketopiperazine were treated with a small amount of aqueous potassium dichromate. The orange color persisted for two days at room temperature. The solution was then acidified with a drop of sulfuric acid. The solution rapidly acquired a green color. This mixture was neutralized with sodium bicarbonate and filtered. The filtrate was acidified with acetic acid and treated with ferric chloride. No positive ferric test was obtained.

A few milliliters of a saturated aqueous solution of 2,5-diketopiperazine were stirred with an equal volume of a 10 per cent chloroform solution of perbenzoic acid. After stirring overnight the aqueous phase failed to give a ferric chloride test.

A saturated aqueous solution of 2,5-diketopiperazine was treated with a small amount of sodium perborate. After remaining at room temperature for several hours an aliquot was treated with ferric chloride. A precipitate formed, but excess ferric chloride failed to give a positive ferric test. The main solution was acidified with acetic acid and was permitted to stand overnight. Treatment with excess ferric chloride gave a negative ferric test.

One gram of 2,5-diketopiperazine, potassium permanganate (1.38 g., 1 equiv.) and 2 g. of potassium bicarbonate were dissolved in 100 ml. of cold water. This solution was refrigerated overnight. The next morning the magenta color had been discharged. The precipitated manganese dioxide was removed by filtration. An aliquot of the colorless solution was acidified with acetic acid and treated with ethanolic ferric chloride.

A red-brown color developed. The bulk of the solution was evaporated in vacuo to dryness. The white residue was washed with hot ethanol. The ethanol washings did not give a distinctive color with ferric chloride. The ethanol-insoluble residue, upon solution in water and acidification with acetic acid, also failed to give a distinctive color with ferric chloride.

2,5-Diketopiperazine (1.14 g., 0.01 mole) was dissolved in 20 ml. of glacial acetic acid. Commercial 40 per cent peracetic acid (3.8 g., 0.02 mole) was added and the solution permitted to remain at room temperature for two days. An aliquot of this mixture gave an intense red-brown color when treated with ethanolic ferric chloride. Paper chromatography, using butanol saturated with water as the mobile phase, indicated two ferric-positive compounds were present. No attempt was made to isolate these compounds.

Attempt to Prepare 1,4-Dihydroxy-2,5-diketopiperazine  
by Ring Closure Methods

2-Bromopropionyl bromide (10.8 g., 0.05 mole) was added dropwise with cooling to a solution of 14 g. (0.2 mole) hydroxylamine hydrochloride in 75 ml. of pyridine. After the addition was complete the reaction mixture was permitted to stand at room temperature overnight. The excess pyridine was removed in vacuo using an efficient vacuum pump. The residue, consisting of oily crystals, appeared to be inseparable by the usual crystallization methods. A paper chromatogram of this material, developed with ethanolic ferric chloride, indicated two ferric-positive

compounds were present. Effective separation of this mixture by means of ion exchange resins was unsuccessful. An apparently highly concentrated fraction was obtained by adsorbing the acidic components with a large excess of the strongly basic IRA-400 (hydroxide phase) resin. The resin was washed until the wash water was nearly neutral and then carbon dioxide bubbled into a slurry of the resin until the pH was lowered to five. The column was then permitted to settle and was washed. Evaporation of the solution of compounds eluted by carbonic acid gave a brown syrup. This syrup gave a very intense ferric test. The attempted preparation of solid derivatives of this material, including hydrochloride, picrate and oxalate salts, failed. Treatment of a portion of this material with a small amount of chloroacetyl chloride resulted in an exothermic reaction; the product proved to be an intractable tar.

Reduction of  $\alpha$ -Nitroesters to  $\alpha$ -Hydroxylamino Esters  
and Attempts to Cyclize These Compounds into  
1,4-Dihydroxy-2,5-diketopiperazines

Ethyl  $\alpha$ -nitropropionate was prepared in good yield using the method of Kornblum (18).

A one-gram sample of ethyl  $\alpha$ -nitropropionate dissolved in 50 ml. of ethanol was hydrogenated at atmospheric pressure and room temperature. One hundred milligrams of five per cent palladium on carbon was used as catalyst. The reduction was stopped after 380 ml. (1.92 equiv.) of hydrogen had been absorbed. The catalyst was removed by filtration and the solvent evaporated in vacuo to yield a colorless syrup. This syrup

was noted to darken with time. A small sample of the syrup failed to give a positive ferric test. It rapidly formed a silver mirror when treated with ethanolic silver nitrate.

The syrup was dissolved in a small amount of water and an equal volume of a saturated aqueous solution of picric acid was added. The excess picric acid was extracted with one portion of benzene and the aqueous phase upon cooling yielded 250 mg. of yellow crystals identified as alanine ethyl ester picrate by comparison of the melting point and the infrared spectrum with those of an authentic sample of this compound.

A variety of modifications were applied to the reduction procedure. Catalysts used were platinum on carbon, palladium on barium sulfate, palladium on calcium carbonate, Raney nickel, and rhodium on alumina. Solvents employed included ethanol, ethyl acetate, ethanolic oxalic acid, and glacial acetic acid. Preparation of a number of solid derivatives was also attempted; these included hydrochlorides, oxalates, picrates, and phenyl urethanes. In all cases the product isolated, if any, was the alanine ethyl ester derivative.

The rate of reduction of ethyl  $\alpha$ -nitropropionate with palladium on barium sulfate was noted to decrease considerably as two equivalents of hydrogen were absorbed. The syrup obtained by the usual isolation procedure was divided into three equal portions. One portion was dissolved in 10 ml. of saturated ethanolic ammonia and permitted to stand overnight at room temperature. The second portion was dissolved in a dilute solution of sodium ethoxide in ethanol and permitted to stand

overnight. The third portion was heated overnight on a steam bath. Only the portion which was treated with sodium ethoxide was found to give a positive test when treated with ferric chloride. The ferric test was quite feeble and no attempt was made to isolate the compound.

Ethyl nitroacetate was prepared in fair yield using the method of Feuer (19). It was also subjected to a number of catalytic reduction procedures. The results were analogous to the above; glycine ethyl ester derivatives were the only pure compounds isolated.

Ethyl  $\alpha$ -nitropropionate was also reduced using the method of Bickel (20). Ethyl  $\alpha$ -nitropropionate (7.35 g., 0.05 mole) and ammonium chloride (2.17 g., 0.05 mole) were dissolved in 50 ml. of aqueous ethanol. With magnetic stirring zinc dust (6.54 g., 0.1 mole) was added. The slurry was stirred 18 hours at room temperature. The solids were removed by filtration and the clear solution evaporated in vacuo to yield a colorless oil containing a white solid. The solids were removed by filtration and were found to consist mostly of ammonium chloride. The remaining syrup, which did not give a ferric test, could not be crystallized. Attempts to prepare the oxalate and hydrochloride salts also failed to yield a crystalline derivative.

### 3-Methyl-2,5-diketopiperazine

This compound was prepared utilizing a modification of the method of Fischer and Otto (15). Alanine ethyl ester hydrochloride (7.65 g., 0.05 mole) was dissolved in about ten milliliters of water. Fifty milliliters of ether and a drop of phenolphthalein were added to this solution

and the resulting mixture placed in a flask which was cooled in an ice-salt bath. Efficient stirring was accomplished by a magnetic stirrer. Two addition funnels were arranged so that their contents could be added simultaneously to the reaction mixture. One funnel contained 5.65 g. (0.05 mole) of chloroacetyl chloride in ca. 10 ml. of dry ether, the other a solution of 4 g. (0.1 mole) of sodium hydroxide in a few milliliters of water. With cooling, the sodium hydroxide solution was added until the solution was basic to phenolphthalein. The chloroacetyl chloride solution was then added very slowly, such that the temperature of the reaction mixture did not exceed 0°. Sodium hydroxide solution was added as necessary to maintain the reaction mixture as nearly neutral as possible. When the additions were complete, the mixture remained basic, so it was neutralized with a lump of dry ice. The two layers were separated and the aqueous layer extracted with an additional portion of ether. The ether solutions were combined and dried with anhydrous magnesium sulfate. Evaporation of the ether extract yielded a low melting solid, 7.7 g., 80 per cent of theory, of crude chloroacetyl alanine ethyl ester. This material was dissolved in 70 ml. of a methanolic solution of ammonia, saturated at 0°C. This solution was placed in a high pressure bomb equipped with a glass liner and heated at 100°C for ten hours. The bomb was cooled to 0°C in an ice bath, opened, and the liner removed. The liner was then cooled further in an ice-acetone bath until crystallization was complete. The resulting crystals, 4.25 g., 82 per cent of theory based on chloroacetyl alanine ethyl ester, were found to melt

at 238° as reported by Fischer and Otto for 3-methyl-2,5-diketopiperazine (15). The N.M.R. spectrum of the compound in trifluoroacetic acid solution was recorded and showed absorptions at 8.30  $\tau$  (3H, doublet), 5.52  $\tau$  (3H, complex multiplet) and 1.66  $\tau$  (2H, poorly resolved multiplet). It was found in the course of this experiment that the desired product could be made in comparable yield from bromoacetyl bromide and alanine ethyl ester hydrochloride or  $\alpha$ -bromopropionyl bromide and glycine ethyl ester hydrochloride. The same method was used.

3,6-bis(p-Acetoxybenzylidene)-2,5-diketopiperazine

This compound was prepared by an adaptation of the method of Richardson, Welch and Calvert (14). Four grams of powdered, fused sodium acetate, 1.5 g. of 2,5-diketopiperazine, 4.5 g. of *p*-hydroxybenzaldehyde and 12 g. of acetic anhydride were placed in a 50-ml. flask equipped with a reflux condenser and a magnetic stirrer. This mixture was boiled under reflux for five hours. After cooling the yellow-brown crystalline mixture was broken up with a glass rod and washed by decantation several times with water and then with ethanol. The bright yellow crystals, 3 g., 56 per cent of theory, were collected by filtration. A small sample of this material was recrystallized from a large volume of glacial acetic acid, m.p. 317-322°, dec. The infrared spectrum was recorded (Nujol mull) and showed  $\lambda_{\max}$ . at 3.15, 5.65, 5.95, and 6.15  $\mu$ . A sample of this compound was sent for analysis.

<u>Anal.</u>	$C_{22}H_{18}N_2O_6$	Calc'd:	C, 65.03;	H, 4.46;	N, 6.89
	(406.39)	Found:	C, 64.91;	H, 4.60;	N, 6.86



p-Acetoxybenzaldehyde

Commercial p-hydroxybenzaldehyde (12.2 g., 0.1 mole) was dissolved by heating in acetic anhydride (10.2 g., 0.1 mole) containing five drops of pyridine. This solution was permitted to stand at room temperature overnight. The next morning it was heated on the steam bath for two hours with occasional swirling. Distillation of this mixture at aspirator pressure gave a fraction, 15 g., 91 per cent, boiling at 150-152°. Richter reported p-acetoxbenzaldehyde boils at 264-265°/760 mm. (16). The infrared spectrum was recorded and showed  $\lambda_{\text{max.}}$  at 3.45, 3.68, 5.72, and 5.90  $\mu$ , among others. There were no absorptions in the 2.5-3.2  $\mu$  region.

3-(p-Acetoxybenzylidene)-1,4-diacetyl-6-methyl-2,5-diketopiperazine

A mixture of 2.6 g. of 3-methyl-2,5-diketopiperazine, 1.7 g. of powdered, fused sodium acetate, 3.6 g. of p-acetoxybenzaldehyde, and 12 ml. of acetic anhydride was prepared in a 50-ml. flask. The flask was equipped with a magnetic stirrer and a reflux condenser. This mixture was boiled under reflux with stirring for six hours. At the end of this period the acetic acid and excess acetic anhydride were removed in vacuo, first with a water aspirator and steam bath, then with an efficient vacuum pump. The yellow-brown residue crystallized slowly. This residue was heated and stirred with 200 ml. of chloroform. The suspended sodium acetate was removed by filtration and the chloroform removed in vacuo. The remaining material was dissolved in a mixture of ten milliliters of glacial acetic acid and one milliliter of methanol. Upon cooling

in ice the golden yellow crystals which formed were collected and recrystallized from acetic acid-methanol. Concentration of the mother liquors yielded an additional crop of crystals, which, after recrystallization, were combined with the first crop. The yield was 1.9 g., m.p. 198-202°. This is 26 per cent of theory based on 3-methyl-2,5-diketopiperazine. The infrared spectrum was recorded in chloroform solution and showed  $\lambda_{\text{max}}$  at: 3.3, 5.68, 5.90, 6.14, and 6.26  $\mu$ , among others. The ultraviolet spectrum in ethanol showed  $\lambda_{\text{max}}$  at 226  $m\mu$  ( $\epsilon = 25,000$ ) and 317  $m\mu$  ( $\epsilon = 25,000$ ). The N.M.R. spectrum of a saturated deuterochloroform solution of the compound was recorded and showed absorption at 8.45  $\tau$  (3H, doublet,  $J = 7$  cps.), 7.96  $\tau$  (1H, singlet), 7.70  $\tau$  (3H, singlet), 7.41  $\tau$  (3H, singlet), 4.87  $\tau$  (1H, quartet,  $J = 7$  cps.), 2.86  $\tau$  (singlet), 2.74  $\tau$  (singlet), 2.66  $\tau$  (center of an  $A_2B_2$  system), and 1.92  $\tau$  (broad singlet). A small sample of the compound after further purification was sent for analysis.

<u>Anal.</u>	$C_{18}H_{18}N_2O_6$	Calc'd:	C, 60.33;	H, 5.06;	N, 7.82
	(358.35)				

<u>Anal.</u>	$C_{16}H_{16}N_2O_5$	Calc'd:	C, 60.72;	H, 5.10;	N, 8.85
	(316.34)	Found:	C, 60.12;	H, 5.38;	N, 8.28

### 3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine

One and six-tenths grams of 3-(p-acetoxybenzylidene)-1,4-diacetyl-6-methyl-2,5-diketopiperazine was added to a mixture of 20 ml. of absolute ethanol and 40 ml. of ammonium hydroxide. A bright red solution

resulted after swirling for about 15 minutes. This solution was permitted to stand at room temperature for an additional hour. It was then evaporated to dryness under reduced pressure on a steam bath. The resulting pale yellow crystals were dissolved in a minimum amount of hot absolute ethanol and refrigerated for several days. The crystals, 0.85 g., which had formed were collected by filtration. The mother liquor was concentrated and cooled for several more days. The second crop of crystals, 0.2 g., was combined with the first. Both crops showed m.p.  $248^{\circ}$ . The total was 1.05 g., 100 per cent yield. The infrared spectrum (Nujol) was recorded and showed  $\lambda_{\text{max.}}$  at 3.15, 5.90, 6.20, and 6.30  $\mu$ . The ultraviolet spectrum was recorded (EtOH) and showed  $\lambda_{\text{max.}}$  at 224  $m\mu$  ( $\epsilon = 12,100$ ) and 317  $m\mu$  ( $\epsilon = 18,700$ ). The N.M.R. spectrum of a 50 per cent solution of the compound in trifluoroacetic acid was recorded and showed absorptions at 8.35  $\tau$  (3H, doublet,  $J = 7$  cps.), 5.52  $\tau$  (1H, poorly resolved multiplet), 3.00  $\tau$  (doublet,  $J = 10$  cps.), 2.80  $\tau$  (singlet), 2.68  $\tau$  (doublet,  $J = 10$  cps.), 1.59  $\tau$  (singlet), and 0.8  $\tau$  (broad peak). A small sample of the compound after additional purification was sent for analysis.

<u>Anal.</u>	$C_{12}H_{12}N_2O_3$	Calc'd:	C, 62.08;	H, 5.17;	N, 12.06
	(232.15)	Found:	C, 61.84;	H, 5.27;	N, 12.05

Attempts to Prepare Tetrahydro-2-(4-hydroxybenzyl)-5-  
methyl-4-oxoglyoxaline-2-carboxylic Acid from 3-(p-  
Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine

Approximately 75 ml. of liquid ammonia was condensed in a 100 ml. flask equipped with a stirrer and dry ice condenser. A suspension of 475 mg. of 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine in 20 ml. of methanol was added to the ammonia. A yellow solution resulted. Sodium, 1.5 g., was added in small pieces to the stirred ammonia solution and stirring was continued until all of the sodium had dissolved. The ammonia was permitted to evaporate and the residue dissolved in about 50 ml. water. Saturation of this brown aqueous solution with carbon dioxide caused no precipitation. The solution was acidified with hydrochloric acid. Filtration of the acidic mixture gave an intractable black tar.

A solution of sodium amide in liquid ammonia was prepared by dissolving ca. one gram of sodium in 50 ml. of liquid ammonia and adding a crystal of ferric nitrate. The solution was permitted to boil under reflux until the blue color faded. 3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine (250 mg.) was added to the ammonia solution. A yellow solution resulted which was permitted to boil under reflux for two hours. The ammonia was allowed to evaporate and the residue was dissolved in a small quantity of water. Acidification with hydrochloric acid precipitated a yellow powder which was identified as unchanged 3-(p-hydroxybenzylidene)-6-methyl-2,5-diketopiperazine, by its melting point and infrared spectrum.

3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine (250 mg.) was dissolved in 10 ml. of five per cent sodium hydroxide. This solution was permitted to stand at room temperature for two days. Acidification of this solution with concentrated hydrochloric acid resulted in the isolation of unchanged starting material, identified by its melting point.

#### Ethyl $\gamma$ -Bromobutyrate

This compound was prepared by modification of the procedure of Reckhow and Tarbell (10). Dry butyrolactone (172 g., 2 moles) was dissolved in 500 ml. of absolute ethanol in a two-liter flask. Anhydrous hydrogen bromide was bubbled into the solution with cooling until 378 g. (4.7 moles) had been added. This solution was boiled under reflux on a steam bath for three hours. The ethyl bromide that had formed was collected by distillation. Sodium-dried benzene was added and the distillation continued until the temperature of the distillate rose to 78°. More hydrogen bromide was added and the reaction mixture was allowed to remain at room temperature overnight. The next day it was distilled. A rather large forerun was collected, which, because of its boiling point, 55°/30 mm., was thought to be unchanged lactone. Continued distillation gave a large fraction, b.p. 75-82°/8 mm. Reckhow and Tarbell report the boiling point of ethyl  $\gamma$ -bromobutyrate to be 76-78°/7 mm. (10). An infrared spectrum of the material was obtained. Absorption at 5.65  $\mu$  indicated the product was contaminated with butyrolactone. A pure product was obtained by extracting the butyrolactone with three large

portions of water. Approximately one-third of the material was lost mechanically. The yield of ethyl  $\gamma$ -bromobutyrate after drying over anhydrous magnesium sulfate was 70 g., 21 per cent of theory.

Reaction of Ethyl  $\gamma$ -Bromobutyrate with Methyl Magnesium Halides

In a 250-ml. three-neck flask equipped with a reflux condenser, a mechanical stirrer, and an addition funnel was placed 100 ml. of sodium-dried ether and five grams (0.206 mole) of magnesium turnings. A small crystal of iodine and a few milliliters of methyl iodide were added. The flask was cooled in an ice bath when obvious reaction had started and the remainder of the methyl iodide, totalling 30 grams (0.206 mole) was slowly added. Stirring was continued until all of the magnesium had dissolved. Twenty grams (0.103 mole) of ethyl  $\gamma$ -bromobutyrate, diluted with an equal volume of dry ether, was added dropwise to the black solution. Only a very small heat effect was noted. After stirring at room temperature for four hours the reaction mixture was poured into an ice cold solution of ammonium chloride, ca. 30 g., in ca. 500 ml. of water. Vigorous evolution of gas was noted. This mixture was extracted with three portions of ether, which were combined and dried with anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo. A yellow oil resulted that showed an infrared spectrum with  $\lambda_{\text{max.}}$  at 2.9 and 5.73  $\mu$ . Distillation of the above oil at 28 mm. resulted in the isolation of only a small quantity of unchanged ester, identified by its boiling point and infrared spectrum.

The same procedure was repeated on a smaller scale except that the reaction mixture was stirred for 20 hours. The results were similar.

Vapor phase chromatography of the product indicated at least four compounds were present.

One mole of methyl magnesium bromide was prepared by the cited method with magnesium (24 g., 1 mole) in 400 ml. of dry ether. The reflux condenser was replaced by a dry ice condenser. The methyl bromide (100 g., 1.18 moles) was added as a gas after distillation through a calcium chloride tube. Fifty grams of ethyl  $\gamma$ -bromobutyrate, diluted with an equal volume of ether, was added over a half hour period. Only a small heat effect was noted. After stirring four hours at room temperature the product, 38 g., was isolated by hydrolysis with aqueous ammonium chloride and extraction with ether. Distillation of this material gave a substance, b.p. 110-118°/30 mm., with an infrared spectrum which showed  $\lambda_{\text{max}}$  at 3.39, 6.90, and 7.90  $\mu$ , but no absorption in the regions 2.5-3.3, 5.10-6.6  $\mu$ .

#### 4-Methyl-1,4-dihydroxypentane

Methyl magnesium iodide was prepared by the slow addition of methyl iodide (152 g., 1.05 moles) to a slurry of magnesium (26 g., 1.08 moles) in 250 ml. sodium-dried ether. The reaction was carried out in a one-liter, three-neck flask equipped with a mechanical stirrer, reflux condenser, and addition funnel. The reaction was protected from moisture and cooled as necessary in an ice bath. When virtually all of the magnesium had dissolved, butyrolactone (43 g., 0.5 mole), dissolved in an equal volume of dry ether was added dropwise with vigorous stirring. A large heat effect was noted, each drop of butyrolactone

striking the surface of the reagent with a hissing sound. After the addition was complete the reaction was stirred for four hours at room temperature. The gray, rather viscous, reaction mixture was poured slowly with stirring into a mixture of water, ca. 200 ml., concentrated sulfuric acid, ca. 10 ml., ammonium chloride, 100 g., and ice, ca. 500 g. This was carried out in a four-liter beaker. The resulting solution was extracted with four 200-ml. portions of ether. Upon evaporation of the dried ether extract only one or two grams of brown oil resulted. The aqueous phase of the extraction was continuously extracted with methylene chloride for two days. The methylene chloride layer was collected and dried with anhydrous sodium sulfate. After filtration and evaporation of the solvent the resulting syrup was distilled, b.p. 120-122°/10 mm. The boiling point of 4-methyl-1,4-dihydroxypentane reported by Ruzicka and Liguori was 120°/12 mm. (11). The yield was 48 g., 82 per cent. The infrared spectrum of the compound was recorded and showed  $\lambda_{\text{max.}}$  at 2.95  $\mu$ . There was no absorption in the region 5-6.4  $\mu$ . The N.M.R. spectrum of a solution of the compound in deuterochloroform was obtained and showed absorption at 8.78  $\tau$  (6H, singlet), 8.45  $\tau$  (4H, poorly resolved multiplet), 6.40  $\tau$  (2H, triplet,  $J = 9$  cps.) and 5.62  $\tau$  (2H, singlet).

#### 4-Methyl-1,4-dibromopentane

This compound was prepared by the method of Ruzicka and Liguori (11). Commercial 48 per cent hydrogen bromide, 20 ml., was saturated with anhydrous hydrogen bromide at 0°C. The yield was 28 ml. Ice cold



4-methyl-1,4-dihydroxypentane, 5 g., was dissolved in the ice cold hydrogen bromide solution, protected from light, and allowed to warm to room temperature. After 24 hours at room temperature the reaction mixture was poured into six volumes of ice water. The resulting 4-methyl-1,4-dibromopentane was extracted with chloroform. Evaporation of the chloroform extract, after washing with dilute sodium bicarbonate and water and drying with anhydrous sodium sulfate, yielded 10.2 g. of brown oil. The material was distilled, b.p.  $88-91^{\circ}/13.5$  mm., yielding 8.5 g. of colorless oil, 82 per cent of theory. The boiling point reported by Ruzicka and Liguori for 4-methyl-1,4-dibromopentane was  $82-85^{\circ}/12$  mm. The infrared spectrum of this compound was recorded and showed no absorption in the region  $2.5-3.2$  and  $3.5-6.6$   $\mu$ . The N.M.R. spectrum of the pure liquid was also recorded; it showed absorption at  $8.27 \tau$  (6H, singlet),  $8.00 \tau$  (4H, complex multiplet) and  $6.57 \tau$  (2H, triplet,  $J = 6$  cps.).

This compound was also prepared by keeping 4-methyl-1,4-dihydroxypentane saturated with anhydrous hydrogen bromide at room temperature for 24 hours. This was accomplished by placing the glycol in a flask which was immersed in a large water bath. Hydrogen bromide was slowly bubbled into the diol with magnetic stirring until saturation was accomplished. The stream of gas was then greatly reduced, but continuously added with stirring for 24 hours. The resulting dibromide was isolated as before. The yields were comparable, if not slightly better.

Dehydrohalogenation of 4-Methyl-1,4-dibromopentane  
with Potassium Benzoate

A modification of the method of Ruzicka and Liguori was employed (11). Potassium benzoate was prepared by dissolving two moles of benzoic acid and one mole of potassium carbonate in a minimum quantity of aqueous ethanol. The solvent was removed by evaporation on a steam bath, the product powdered, and then dried at 130°.

A solution of potassium benzoate (61 g., 0.38 mole) in 75 ml. of glacial acetic acid, to which a few milliliters of water had been added, was prepared by stirring and heating to 100°. 4-Methyl-1,4-dibromopentane (93 g., 0.38 mole) was added in one portion. The reaction mixture became quite thick with a white precipitate. Stirring and heating at 100° was continued for a half an hour. At the end of this period the mixture was poured into a large volume of ice water, ca. two liters, in a four-liter beaker. This solution was neutralized by the addition of powdered solid sodium bicarbonate and extracted with chloroform. The chloroform solution was dried with anhydrous sodium sulfate and the solvent evaporated in vacuo. The residue, upon distillation at 70°/60 mm. yielded 30 g., 48 per cent, of the material identified by Ruzicka and Liguori as 1-bromo-4-methyl-3-pentene. They reported a boiling point of 155°/760 mm. (11). The average yield of three reactions conducted in this manner was 46 per cent. The infrared spectrum of the distillate indicated the presence of a contaminant containing a carbonyl group. This contaminant was removed completely by chromatography over a short

alumina column which was eluted with petroleum ether. Vapor phase chromatography of the resulting material on a 300 foot petroleum grease capillary column indicated that two compounds with nearly the same retention time were present in the ratio of 80 : 20. The infrared spectrum showed no absorption in the region 2.5-3.1  $\mu$  and 3.7-6.0  $\mu$ . Absorption was noted at 3.26, 6.08, and 11.20  $\mu$ . The N.M.R. spectrum of the liquid was recorded and showed absorption, among others, at 5.30  $\tau$  (2.2H, singlet) and 4.90  $\tau$  (3H, triplet,  $J = 7$  cps.).

Continued distillation of the original reaction product yielded 12 g., 14 per cent, of material, b.p. 110°/25 mm. The infrared spectrum of this compound showed  $\lambda_{\text{max.}}$  at 3.34, 5.73, and 7.95  $\mu$ . There were no strong absorptions in the region 11.0 to 15.0  $\mu$ . The N.M.R. spectrum of this material was recorded and showed absorption at 8.59  $\tau$  (6H, singlet), 8.10  $\tau$  (7H, poorly resolved multiplet) and 6.58  $\tau$  (2H, triplet,  $J = 6$  cps.).

The same procedure was employed as in the method cited with the exception that water was rigorously excluded and the solvent acetic acid contained acetic anhydride, five per cent by volume. The average yield of three reactions carried out in this manner was 61 per cent. The by-product was formed in about 14 per cent yield.

#### Dehydrohalogenation of 4-Methyl-1,4-dibromopentane

##### with Alcoholic Potassium Hydroxide

A solution of 4-methyl-1,4-dibromopentane (24.4 g., 0.1 mole) in 50 ml. of absolute ethanol was prepared. With magnetic stirring, a

solution of potassium hydroxide (5.6 g., 0.1 mole), in 50 ml. of absolute ethanol was added over a three hour period. After stirring for 18 hours at room temperature the reaction mixture was neutral to phenolphthalein. The precipitated potassium bromide was collected by filtration and the ethanol solution distilled. There was no fraction with the boiling range corresponding to the desired 1-bromo-4-methyl-3-pentene. This experiment was repeated with the same result.

Alkylation of Ethyl Acetoacetate with 1-Bromo-  
4-methyl-3-pentene

The procedures used were adapted from Organic Synthesis (12).

Dry ethanol was prepared by boiling commercial absolute ethanol under reflux with two per cent by weight magnesium turnings for six hours. One hundred milliliters of this ethanol was distilled directly into a three neck flask which was equipped with an addition funnel, a mechanical stirrer, and a reflux condenser. The entire apparatus was carefully protected from moisture. Five and three tenths grams (0.23 mole) of sodium metal was added to the ethanol and stirring started. When the solution of the sodium was complete the mixture was heated to boiling. Ethyl acetoacetate (30 g., 0.23 mole) was added to this hot solution and, after it was stirred a few minutes, the addition of 1-bromo-4-methyl-3-pentene was started. A total of 30 g. (0.186 mole) of this compound was added over a period of about two hours. The heating and stirring were continued for 20 hours. The solution was then cooled and carefully neutralized with glacial acetic acid. The

precipitate was allowed to settle and the liquid decanted. The solids were washed with several portions of absolute ethanol by decantation. The washings were added to the main solution. After the ethanol had been removed by evaporation at aspirator pressure the residual oil was distilled. The major fraction weighed ten grams, 31 per cent of the theoretical yield, and had a boiling point of  $65^{\circ}/3.8$  mm. This compound was carefully redistilled and a portion sent for analysis.

<u>Anal.</u>	$C_{10}H_{18}O_2$	Calc'd:	C, 70.70;	H, 10.61
	(170.25)	Found:	C, 70.92;	H, 11.32

Vapor phase chromatography indicated a single compound was present. The infrared spectrum was recorded and showed  $\lambda_{\max}$  at 3.20, 3.35, 5.76, 6.05, and 11.25  $\mu$ , among others. The ultraviolet spectrum of a solution of 3.2 mg. of this material in 100 ml. of 95 per cent ethanol showed only end absorption. The N.M.R. spectrum of the pure liquid was recorded and showed absorption at 8.82  $\tau$  (3H, triplet,  $J = 7$  cps.), 8.52 $\tau$  (singlet showing fine structure), 7.6-8.25  $\tau$  (six rather broad peaks), 5.96  $\tau$  (2H, quartet,  $J = 7$  cps.), 5.37  $\tau$  (2.1 integration units, singlet), and 4.90  $\tau$  (4.9 integration units, triplet,  $J = 7$  cps.).

Sodium (2.3 g., 0.1 mole) was dissolved in 50 ml. of dry ethanol. Ethyl acetoacetate (13 g., 0.1 mole) was added to the hot sodium ethoxide solution followed by the slow addition of 1-bromo-4-methyl-3-pentene (23 g., 0.14 mole). This reaction mixture became neutral after boiling for seven hours under reflux. The product (16 g., 0.075 mole) isolated by distillation was found to boil at  $75-78^{\circ}/0.3$  mm. This material gave

a dark green ferric chloride test. The infrared spectrum showed absorption at 5.72, 5.81, 6.12, and 11.25  $\mu$ . The N.M.R. spectrum of this compound was recorded and showed absorption at 8.78  $\tau$  (3H, triplet,  $J = 7$  cps.), 7.9-8.6  $\tau$  (several peaks), 7.85  $\tau$  (3H, singlet), 6.53  $\tau$  (1H, triplet,  $J = 6$  cps.), 5.83  $\tau$  (2H, quartet,  $J = 7$  cps.), 5.32  $\tau$  (1H, singlet), and 4.85  $\tau$  (0.5 H, triplet,  $J = 7$  cps.). A small sample of this compound was purified by careful redistillation and sent for analysis.

<u>Anal.</u>	$C_{12}H_{20}O_3$	Calc'd:	C, 67.90;	H, 9.49
	(212.29)	Found:	C, 68.10;	H, 9.27

Reduction of Ethyl 2-Acetyl-6-methyl-5-heptenoate  
with Sodium Borohydride

Fifty grams (0.236 mole) of pure, redistilled ethyl 2-acetyl-6-methyl-5-heptenoate was dissolved in 360 ml. of 95 per cent ethanol. This solution, contained in a 500-ml. flask, was cooled in an ice bath and stirred magnetically. Powdered, dry sodium borohydride (9.0 g., 0.24 mole) was added as a solid in small portions over a half hour period to the cooled and agitated reaction mixture. Stirring was continued for an additional hour after all of the borohydride had been added. The reaction mixture was poured into one liter of 50 per cent saturated sodium chloride solution. This solution was extracted thoroughly with ether. The ether extracts were combined and washed with one portion of water. Evaporation of the solvent in vacuo after drying with anhydrous sodium sulfate yielded 48 g. (0.223 mole), 95 per cent yield, of a cloudy colorless syrup. A small sample of this

material was purified by careful distillation through an efficient column, b.p. 90°/0.8 mm., and sent for analysis.

<u>Anal.</u>	$C_{12}H_{22}O_3$	Calc'd:	C, 67.25;	H, 10.35
	(214.31)	Found:	C, 67.74;	H, 9.99

The infrared spectrum of this compound showed  $\lambda_{\max}$  at 2.90, 3.22, 3.40, 5.74, 6.05, and 11.25  $\mu$ . The N.M.R. spectrum of the pure liquid was recorded and showed absorption, among others, at 6.11  $\tau$  (1H, singlet), 5.81  $\tau$  (2H, quartet,  $J = 7$  cps.), 5.27  $\tau$  (1.4H, singlet), and 4.83  $\tau$  (0.7H, triplet,  $J = 6$  cps.).

#### Dehydration of Ethyl 2-(1-Hydroxyethyl)-6-methyl-5-heptenoate

Ethyl 2-(1-hydroxyethyl)-6-methyl-4-heptenoate, two grams, was dissolved in 25 ml. of sodium-dried benzene containing 25 mg. of iodine. This mixture was placed in a one-neck 50-ml. flask equipped with a Dean-Stark apparatus. There was no water visible in the collector after 24 hours' boiling under reflux. The infrared spectrum of the reaction mixture showed absorption at 2.90 and 5.74  $\mu$ , indicating no reaction had occurred. This experiment was repeated three times with the following modifications: twenty-five milliliters of benzene and 250 mg. of iodine were used, as were 25 ml. of m-xylene and 250 mg. of iodine. Twenty-five milliliters of decalin and 100 mg. of iodine boiled under reflux for four hours were also tried. In all cases the infrared spectrum appeared to be unchanged.

Ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate, two grams, was boiled under reflux for one hour in a solution of two grams of phosphorus

oxychloride in 20 ml. of toluene. After cooling to room temperature the reaction mixture was stirred for a half hour with 50 ml. of a saturated solution of sodium bicarbonate. The organic layer was collected, washed with water and dried with anhydrous sodium sulfate. The solvent was removed in vacuo and the residue chromatographed over a short alumina column, which was eluted with chloroform. Four fractions were collected. Vapor phase chromatography indicated three of these fractions to be quite pure and contain the same compound. The infrared spectrum of this material did not show absorption in the region of  $2.9\ \mu$ , but did show a band at  $5.77\ \mu$ . The N.M.R. spectrum of a carbon tetrachloride solution was recorded; it was very complex and showed only very slight absorptions in the region associated with olefinic protons.

A solution of one gram of ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate and 0.1 gram of p-toluenesulfonic acid in 10 ml. of benzene was boiled under reflux for three hours. The infrared spectrum was recorded and showed  $\lambda_{\text{max.}}$  at  $2.9$  and  $5.75\ \mu$ .

An ice cold solution of 2.4 g. (0.01 mole) of ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate in 1.6 g. (0.02 mole) of pyridine was prepared. Thionyl chloride, 1.19 g., (0.01 mole) was slowly added, with cooling in ice, to this solution. The reaction mixture was permitted to warm to room temperature after the addition was complete. After remaining for an hour it was poured on a mixture of water and cyclohexane. This mixture was shaken and the organic layer collected. The cyclohexane solution was dried with anhydrous sodium sulfate and placed directly on a short alumina column. Elution of this column



with 50 per cent chloroform-cyclohexane gave about two grams of pale yellow oil. The infrared spectrum showed no absorption in the  $2.9\ \mu$  region, but a strong band at  $5.77\ \mu$  was observed. The N.M.R. spectrum of the liquid was recorded. This spectrum was very similar to that of ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate except that the absorption assigned to hydroxyl,  $6.11\ \tau$ , was absent. The oil was dissolved in ten milliliters of pyridine and permitted to stand at room temperature overnight. The next morning the pale brown solution was poured into 1 N sulfuric acid and extracted with cyclohexane. The extracts were combined, washed with water and dried with anhydrous sodium sulfate. The infrared spectrum of this solution showed, among others, bands at  $5.77\ \mu$  and  $5.83\ \mu$  of nearly equal intensity. The cyclohexane was removed in vacuo and the residue dissolved in a few milliliters of quinoline. The quinoline solution was heated for ten minutes at  $200^\circ$ . Upon cooling this mixture was dissolved in chloroform and extracted with 1 N sulfuric acid, sodium bicarbonate solution, and water. The chloroform solution was dried with anhydrous sodium sulfate and passed over a short alumina column which was eluted with chloroform. The effluent solvent was evaporated; the infrared spectrum showed no change in the proportions of the  $\lambda_{\text{max}}$  of the carbonyl groups. Vapor phase chromatography indicated that at least six compounds were present.

Dry, crude ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate (48 g., 0.224 mole) and p-toluenesulfonyl chloride (61 g., 0.32 mole) were dissolved in 250 ml. of dry pyridine. Crystals were observed to form after about an hour. This mixture was permitted to remain at room temperature

with occasional swirling for 48 hours. At the end of this period 100 g. of ice was added and the mixture was stirred for half an hour. It was then poured on a mixture of 300 g. of concentrated hydrochloric acid and 1,000 g. of ice water. This mixture was extracted with four portions of ether which were in turn extracted with one portion of water. The ether solution was thoroughly dried with anhydrous magnesium sulfate. Evaporation of the solvent in vacuo, first with the water aspirator then with an efficient vacuum pump yielded 81.7 g. of a viscous yellow oil. The theoretical yield was 71 g. A solution of 25 g. of sodium in 500 ml. of absolute ethanol was prepared and the above oil added to it. This mixture became quite pasty with a white precipitate, most of which soon redissolved. This solution was stirred for 20 minutes at room temperature and then poured slowly, with stirring, into a slurry of about 500 g. of crushed dry ice in two liters of water. When all of the dry ice had evaporated and the solution contained no ice it was thoroughly extracted with ether. The ether extracts were washed with one portion of water and then dried with anhydrous magnesium sulfate. Removal of the ether in vacuo yielded 44.3 g. of a light yellow oil containing some suspended solid. The theoretical yield was 43.9 g. The infrared spectrum of this material was recorded and showed  $\lambda_{\text{max.}}$  at: 3.26, 3.42, 5.83, 6.05, and 11.23  $\mu$ . The N.M.R. spectrum of the crude liquid also was recorded and showed absorptions at 8.78  $\tau$  (triplet,  $J = 7$  cps.), 8.76  $\tau$  (triplet,  $J = 7$  cps.), 7.5-8.5  $\tau$  (about ten peaks), 5.85  $\tau$  (quartet,  $J = 7$  cps.), 5.82  $\tau$  (quartet,  $J = 7$  cps.), 5.28  $\tau$  (2.8 integration units, singlet), 4.87  $\tau$  (1.3 integration units,

poorly resolved triplet), 4.02  $\tau$  (19 integration units, quartet,  $J = 7$  cps.), and 3.14  $\tau$  (20 integration units, quartet,  $J = 7$  cps.).

Separation of *Cis*- and *Trans*-Ethyl 2-Ethylidene-6-  
methyl-5-heptenoate

A chromatographic column 90 mm. in diameter and 154 cm. long containing Merck acid-washed alumina was prepared. The solvent employed was dry redistilled benzene. The finished column contained 21 pounds of alumina and 6.6 liters of benzene. The crude ethyl 2-ethylidene-6-methyl-5-heptenoate, 44.3 g., was placed on the column and eluted with benzene. The first material was noted to be eluted after about seven liters of solvent had been collected. Fourteen fractions of about 400 ml. each were then collected. These fractions, after removal of the solvent, weighed respectively: 0.8, 1.3, 2.2, 2.2, 2.5, 3.1, 4.3, 5.5, 3.1, 1.6, 1.5, 0.8, 1.0, and 0.4 g. The total weight obtained from the column was 30.3 g., 69 per cent of theory based on the ethyl 2-(1-hydroxyethyl)-6-methyl-5-heptenoate used. The N.M.R. spectrum of selected fractions was recorded. Fractions 1, 2, 3, and 4 showed identical spectra with the following absorptions: 8.78  $\tau$  (triplet,  $J = 7$  cps.), 7.5-8.5  $\tau$  (about 7 peaks), 5.82  $\tau$  (quartet,  $J = 7$  cps.), 5.28  $\tau$  (singlet), 4.87  $\tau$  (poorly resolved triplet) and 4.01  $\tau$  (quartet,  $J = 7$  cps.). Fractions 11, 12, 13, and 14 were also identical and showed absorptions at 8.78  $\tau$  (triplet,  $J = 7$  cps.), 7.8-8.5  $\tau$  (about 7 peaks), 5.83  $\tau$  (quartet,  $J = 7$  cps.), 5.28  $\tau$  (singlet), 4.87  $\tau$  (poorly resolved triplet), and 3.12  $\tau$  (quartet,  $J = 8$  cps.). The other fractions appeared to be

mixtures of these two compounds. It was also noted that these two compounds could be separated by vapor phase chromatography. The most effective separation was noted at 175° using silacone grease as the stationary phase.

Three of the middle fractions were combined and distilled, b.p. 70°/1.1 mm. A sample of the distillate which showed an infrared spectrum with  $\lambda_{\text{max}}$  at 3.26, 3.42, 5.83, 6.05, and 11.23  $\mu$  was sent for analysis.

<u>Anal.</u>	$\text{C}_{12}\text{H}_{20}\text{O}_2$	Calc'd:	C, 73.43;	H, 10.27
	(196.29)	Found:	C, 73.22;	H, 10.34

#### Attempts to Isomerize the Ethylidene Double Bond of

##### Ethyl 2-Ethylidene-6-methyl-5-heptenoate

A small sample, ca. one gram of ethyl 2-ethylidene-6-methyl-5-heptenoate, which showed absorption in the N.M.R. spectrum at 3.12  $\tau$  and 4.01  $\tau$ , of relative areas of 70 and 30 units, was dissolved in 25 ml. of dilute sodium ethoxide in dry ethanol. After half an hour at room temperature this reaction mixture was poured into a slush of dry ice and water. When the dry ice had evaporated and the ice melted the solution was thoroughly extracted with ether. The ether extracts were dried with anhydrous magnesium sulfate and the solvent removed in vacuo. An N.M.R. spectrum of the residue indicated the relative proportions of the areas of the absorptions at 3.12  $\tau$  and 4.01  $\tau$  had not been changed.

Approximately 0.5 g. of ethyl 2-ethylidene-6-methyl-5-heptenoate, which had an N.M.R. spectrum showing absorption at 3.12  $\tau$  and 4.01  $\tau$  of relative areas at 70 and 30 units, was dissolved in two milliliters of

cyclohexane. This solution was placed in a quartz cell and irradiated with a General Electric model 51 sun lamp at a distance of eight inches. N.M.R. spectra of the solution were recorded after 1.5 and 16 hours. They showed no change in the relative areas of the absorptions at  $3.12 \tau$  and  $4.01 \tau$ .

A sample, ca. 500 mg. of ethyl 2-ethylidene-6-methyl-5-heptenoate, which showed absorption at  $3.12 \tau$  and  $4.01 \tau$  of relative areas of 70 and 30 units in the N.M.R. spectrum, was dissolved in ten milliliters of ten per cent anhydrous hydrogen bromide in dry ether at  $0^\circ$ . After five minutes at  $0^\circ$  the solvent was removed in vacuo without heating. An N.M.R. spectrum of the resulting brown oil was recorded. It showed only very slight absorptions in the region,  $2.0-6.0 \tau$ .

Reduction of Cis- and Trans-Ethyl 2-Ethylidene-6-methyl-5-heptenoate with Lithium Aluminum Hydride

One gram (0.0264 mole) of lithium aluminum hydride was magnetically stirred with 50 ml. of sodium-dried ether in a stoppered 200-ml. flask for 20 minutes. This solution was then cooled in an ice bath and 4.4 g. (0.022 mole) of ethyl 2-ethylidene-6-methyl-5-heptenoate in 25 ml. of dry ether was added over a period of half an hour. The reaction was stirred in the ice bath for an additional hour. Excess lithium aluminum hydride was destroyed by the slow addition of a few milliliters of ethyl acetate. The ether solution was poured into 400 ml. of ice cold 1 N sulfuric acid. The ether layer was separated and the aqueous layer extracted with three additional portions of ether. The ether

extracts were combined and dried over anhydrous potassium carbonate. Evaporation of the solvent yielded 3.4 g., (theory 3.4 g.) of a colorless syrup.

This crude material was chromatographed using a silicic acid column prepared from 130 g. of silicic acid and stock chloroform. Elution with three column-volumes of stock chloroform gave 0.4 g. of material which had an odor similar to citronellol. The infrared spectrum of this material was recorded and showed  $\lambda_{\text{max.}}$  at 3.23, 3.40, 3.65, 5.82, 6.05, and 11.25  $\mu$ . The N.M.R. spectrum of the undiluted liquid was recorded and showed absorptions at: 9.08  $\tau$  (triplet,  $J = 7$  cps.), 8.8-6.6  $\tau$  (complex, about seven peaks), 5.30  $\tau$  (singlet), 4.90  $\tau$  (poorly resolved triplet), and 1.25  $\tau$  (doublet,  $J = 2.3$  cps.).

Continued elution of the silicic acid column with ten per cent by volume ethanol in chloroform gave 2.5 g., 83 per cent, of a colorless syrup, referred to as mycelianol. The infrared spectrum of this material was recorded and showed  $\lambda_{\text{max.}}$ , among others, at 3.0, 3.28, 6.05, and 11.25  $\mu$ . The infrared spectra of this material were almost identical regardless of the isomer reduced.

If the ester reduced was cis-(methyl-carbethoxy) the following absorptions in the N.M.R. spectrum were observed: 8.42  $\tau$  (singlet), 8.30  $\tau$  (singlet showing fine structure), 8.2-7.7  $\tau$  (several peaks), 5.92  $\tau$  (singlet), 5.32  $\tau$  (singlet), 4.91  $\tau$  (poorly resolved triplet), and 4.65  $\tau$  (quartet,  $J = 7$  cps.).

The reduction of the trans-(methyl-carbethoxy) ester led to the observation of the following absorptions on the N.M.R. spectrum:

8.42  $\tau$  (singlet), 8.30  $\tau$  (singlet), 8.2-7.7  $\tau$  (several peaks), 6.04  $\tau$  (singlet), 5.32  $\tau$  (singlet), 4.90  $\tau$  (poorly resolved triplet) and 4.64  $\tau$  (quartet,  $J = 7$  cps.).

When a mixture of the cis- and trans- ester was reduced the N.M.R. spectrum showed absorptions at both 6.04 and 5.92  $\tau$ . In the course of this experiment it was observed that the two isomeric alcohols could be separated by careful silicic acid chromatography. The column was prepared by slurring 50 g. silicic acid per gram of crude product with chloroform and permitting it to settle. The eluting solvent was chloroform. Some separation was observed by noting the N.M.R. absorptions at 6.04 and 5.92  $\tau$  in the eluted material. An attempt was made to separate the isomeric alcohols by chromatography on a short column of powdered anhydrous calcium chloride; the column was eluted with cyclohexane. N.M.R. spectra taken on the eluted fractions indicated no separation was achieved.

#### Geranyl Bromide

This compound was prepared by a modification of the method of Schmitt (13). Pure geraneol (Aldrich), 9 g. (0.053 mole), was dissolved in ten milliliters of cyclohexane to which 0.3 g. pyridine had been added. Seven grams (0.026 mole) of phosphorus tribromide was slowly added to this solution with efficient stirring and cooling in an ice bath. The mixture was cooled for one hour after the addition was complete and then permitted to warm to room temperature and stand for 12 hours. The reaction mixture was poured into 100 ml. of ice water,

agitated, and the cyclohexane layer collected. This organic phase was washed with 100 ml. of dilute sodium bicarbonate solution and then washed with water. It was dried by stirring with a large excess of calcium chloride. Purification of a small portion of this pale yellow, clear cyclohexane solution by alumina chromatography was attempted. A short, acid-washed alumina column was prepared using cyclohexane as solvent. Upon placing the sample on the column a very large evolution of heat was noted, sufficient to boil the cyclohexane, and the top of the column became dark purple in color. The same observation was made using a column prepared from silicic acid and cyclohexane. Chromatography on an anhydrous magnesium sulfate column, while not as violent as the alumina or silicic acid, resulted in the formation of dark-colored materials. The solvent was removed in vacuo from the remaining crude geranyl bromide. The infrared spectrum was recorded and did not show  $\lambda_{\text{max}}$  in the regions 2.5-3.2 and 5.0-6.0  $\mu$ . Bands were observed at 3.42 and 6.05  $\mu$ . The N.M.R. spectrum of the pure liquid was recorded, and showed absorptions, among others, at 8.28  $\tau$  (singlet), 6.06  $\tau$  (doublet), and 4.2-5.2  $\tau$  (several peaks). The yield was not calculated because of the loss during attempted chromatography.

1-Bromo-2-ethylidene-6-methyl-5-heptene

The method used for the preparation of geranyl bromide was also used to prepare this compound. 2-Ethylidene-6-methyl-5-hepten-1-ol, 2.5 g., was reacted with 2.0 g. phosphorus tribromide in a solution of ten milliliters of cyclohexane and two drops of pyridine according to



the method described above. The product 3.0 g., 85 per cent yield, was also isolated in the same manner, without chromatography. A very small sample was noted to react in the presence of alumina. The infra-red spectrum, which was virtually the same regardless of the double bond isomer reacted, was recorded and showed  $\lambda_{\text{max}}$ , among others, at 3.4, 6.05, and 11.25  $\mu$ .

When the cis-(methyl-hydroxymethyl) compound was reacted, the following absorptions in the N.M.R. spectrum were observed: 8.40  $\tau$  (singlet), 8.28  $\tau$  (singlet showing fine structure), 7.6-8.2  $\tau$  (poorly resolved multiplets), 5.98  $\tau$  (12 integration units, singlet), 5.28  $\tau$  (4 integration units, singlet), 4.82  $\tau$  (poorly resolved triplet), and 4.46  $\tau$  (ca. 6 integration units, quartet, showing fine structure,  $J = \text{ca. } 6 \text{ cps.}$ ).

Reaction of the trans-(methyl-hydroxymethyl) compound led to the corresponding bromide which showed N.M.R. absorptions at 8.42  $\tau$  (singlet), 8.28  $\tau$  (singlet, showing fine structure), 7.6-8.2  $\tau$  (several peaks), 5.95  $\tau$  (17 integration units, singlet), 5.22  $\tau$  (2.5 integration units, singlet), 4.78  $\tau$  (7 integration units, poorly resolved triplet), and 4.19  $\tau$  (9 integration units, quartet,  $J = \text{ca. } 7 \text{ cps.}$ ).

#### p-Geranyloxybenzoic Acid

A mixture of ethyl p-hydroxybenzoate (332 mg., 0.002 mole) and powdered anhydrous potassium carbonate (414 mg., 0.003 mole) in 20 ml. of dimethylsulfoxide was prepared. With magnetic stirring at room temperature geranyl bromide (234 mg., 0.0011 mole) was added to this

mixture over a two-hour period. The reaction mixture was stirred for 20 additional hours. The solvent was then removed at moderate temperature by use of an efficient vacuum pump. The residue was extracted with a mixture of ether and water. The ether layer was collected, evaporated to dryness, and heated on a steam bath with three potassium hydroxide pellets dissolved in a minimum amount of aqueous ethanol. After two hours the nearly dry mixture was dissolved in water, filtered, and acidified with hydrochloric acid. The precipitate that formed was dissolved by warming and adding aqueous ethanol. After cooling in ice, the crystals, 197 mg., 65 per cent yield, were collected. The material was found to melt at  $118^{\circ}$  as reported by Birch (5). The N.M.R. spectrum of a five per cent solution of the compound in carbon tetrachloride was recorded and showed absorptions at  $8.1-8.5 \tau$  (three sharp peaks),  $7.7-8.0 \tau$  (poorly resolved multiplet),  $5.41 \tau$  (doublet,  $J = 8$  cps.),  $4.90 \tau$  (poorly resolved triplet),  $4.55 \tau$  (triplet,  $J = 6$  cps.),  $3.08 \tau$  (doublet,  $J = 9$  cps.), and  $1.95 \tau$  (doublet,  $J = 9$  cps.).

A solution of geranyl bromide, 464 mg. (0.002 mole) potassium carbonate, 280 mg. (0.002 mole), in dimethylsulfoxide, 20 ml., was stirred for four hours at room temperature. The reaction mixture was poured into 100 ml. of ice water and extracted with four portions of ether. The aqueous phase was acidified with nitric acid and excess silver nitrate added. The precipitated silver bromide was collected by filtration, washed with water and acetone, and dried. The weight of the dry silver bromide was 384 mg. The theoretical yield, 0.002 moles, was 376 mg.

3-(p-Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine

This compound was prepared using the coupling method employed for the preparation of p-geranyloxybenzoic acid. The yield, however, was very low so a modification of the procedure used by Lauer and Labriola was utilized (17).

3-(p-Hydroxybenzylidene)-6-methyl-2,5-diketopiperazine (464 mg., 0.002 mole) and powdered anhydrous potassium carbonate (280 mg., 0.002 mole) were placed in a flask equipped with a reflux condenser and magnetic stirrer. Stirring was started and ten milliliters of dry acetone was added. This mixture was boiled under reflux and geranyl bromide (652 mg., 0.003 mole), dissolved in five milliliters of dry acetone, was added to the boiling solution over a 2.5 hour period. The reaction mixture was boiled under reflux for an additional five hours. After cooling the solvent was removed in vacuo and the residue extracted with several portions of boiling chloroform. The chloroform solution was filtered and the solvent evaporated in vacuo. The pale yellow residue was recrystallized from a minimum amount of absolute ethanol. The crystals that formed were collected. Upon concentration and cooling the filtrate a second crop was obtained. The total yield was 270 mg., or 37 per cent of theory. The melting point observed on a purified sample was 172°. The infrared spectrum was recorded and is reproduced as Figure 1. The ultraviolet spectrum showed  $\lambda_{\text{max.}}$  at 226 m $\mu$  ( $\epsilon = 17,500$ ), and 317 m $\mu$  ( $\epsilon = 27,500$ ). The N.M.R. spectrum was recorded as a saturated deuteriochloroform solution and is given as Figure 2.

A sample of the material was recrystallized three times, with hot filtration of the solution, from redistilled absolute ethanol. After drying in vacuo at 100° the sample was sent for analysis.

<u>Anal.</u>	$C_{22}H_{28}N_2O_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 71.07;	H, 7.59;	N, 7.25

The sample was recrystallized two more times from absolute ethanol, dried and sent for analysis.

<u>Anal.</u>	$C_{22}H_{28}N_2O_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 70.55;	H, 7.73;	N, 7.51

The sample was then recrystallized once from n-butanol and once from acetone, dried and sent for analysis.

<u>Anal.</u>	$C_{22}H_{28}N_2O_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 71.77;	H, 7.69;	N, 7.60

3-[p-(2-Ethylidene-6-methyl-5-heptenoxy)-benzylidene]-  
6-methyl-2,5-diketopiperazine

The same procedure used for preparation of 3-p-geranyloxybenzylidene-6-methyl-2,5-diketopiperazine was employed for the preparation of this compound. The only exception was the use of 2-ethylidene-6-methyl-5-heptenyl bromide instead of geranyl bromide.

When the trans-(methyl-bromomethyl) isomer was used, the product 325 mg., 44 per cent, showed the following characteristics: the melting point observed on a purified sample was 169-170°; the infrared

spectrum (KBr) was recorded and is reproduced as Figure 3; the N.M.R. spectrum of a saturated deuterochloroform solution was recorded and is shown in Figure 4; the ultraviolet spectrum (EtOH) showed  $\lambda_{\text{max.}}$  at 227  $\text{m}\mu$  ( $\epsilon = 17,500$ ) and 317  $\text{m}\mu$  ( $\epsilon = 31,200$ ). Silicic acid chromatography of the crude product, utilizing chloroform-ethanol as solvent, did not appear to effect purification. A sample of the material was recrystallized three times, with hot filtration of the solution, from redistilled absolute ethanol. The sample was dried in vacuo at  $100^\circ$  and sent for analysis.

<u>Anal.</u>	$\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 70.91;	H, 7.78;	N, 7.47

The material was then recrystallized once from n-butanol and once from acetone, dried, and sent for analysis.

<u>Anal.</u>	$\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 69.53;	H, 7.62;	N, 8.67

The use of the cis-(methyl-bromomethyl) isomer as the alkylating agent led to a 42 per cent yield, 310 mg. The observed melting point was  $160\text{--}161^\circ$ . The infrared spectrum (KBr) was recorded and is shown as Figure 5. The N.M.R. spectrum recorded as a saturated deuterochloroform solution is shown in Figure 6. The ultraviolet spectrum showed  $\lambda_{\text{max.}}$  at 226  $\text{m}\mu$  ( $\epsilon = 17,500$ ) and 317  $\text{m}\mu$  ( $\epsilon = 25,500$ ).

A sample of the material was recrystallized three times from redistilled ethanol, dried in vacuo, and sent for analysis.

<u>Anal.</u>	$C_{22}H_{28}N_2O_3$	Calc'd:	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 69.62;	H, 7.89;	N, 7.45

The sample was then recrystallized once from n-butanol and once from acetone, dried and sent for analysis.

<u>Anal.</u>	$C_{22}H_{28}N_2O_3$	Calc'd.	C, 71.71;	H, 7.66;	N, 7.60
	(362.44)	Found:	C, 70.18;	H, 7.47;	N, 8.64

#### Mycelianamide

The physical properties of an authentic sample of mycelianamide furnished by Glaxo Laboratories were investigated. The compound was found to show m.p. 152-160°; [lit. 170-172° dec. (4)]. The N.M.R. spectrum of a deuteriochloroform solution was recorded and is reproduced as Figure 7. The ultraviolet spectrum (EtOH) showed  $\lambda_{\max.}$  at 232  $m\mu$  ( $\epsilon = 15,300$ ) and 320  $m\mu$  ( $\epsilon = 29,600$ ). The infrared spectrum (KBr) was obtained and is shown as Figure 8.

#### Reduction of Mycelianamide

The compound was reduced using essentially the method of Birch for the preparation of deoxymycelianamide.

Natural mycelianamide, 40 mg., m.p. 150-155° [lit. m.p. 170-171° dec. (6)] was dissolved in three milliliters of glacial acetic acid. Zinc dust, 0.3 g., was added in small portions with magnetic stirring over an eight hour period. The mixture was stirred at room temperature overnight. The next morning the zinc residues were removed by filtration and washed with several portions of hot ethanol. The washings

were combined with the main solution and evaporated to dryness. It was noted that the oily residue gave a positive ferric test. Therefore, the residue was redissolved in acetic acid and ca. 0.2 g. of zinc dust added. This mixture was permitted to stand, with occasional swirling, for 24 hours. The zinc was removed and the solution evaporated as before. The residue did not give a ferric test. This material was washed with a little water and dissolved in a small amount of hot ethanol. The ethanol solution was filtered and permitted to cool slowly. The crystals, ca. 4 mg., which formed were collected by filtration and dried. When heated on the melting point apparatus the hexagonal plates were noted to lose birefringence at 165° and become needles. The needles melted at 187°. The infrared spectrum (KBr) of the material was recorded and is reproduced as Figure 9. The ultraviolet spectrum was recorded and showed  $\lambda_{\text{max.}}$  at 317  $m\mu$  ( $\epsilon = 7,900$ ) and 227  $m\mu$  ( $\epsilon = 7,500$ ). The N.M.R. spectrum of one milligram of the compound in deuteriochloroform solution was obtained using the microcell technique. This spectrum is reproduced as Figure 10.

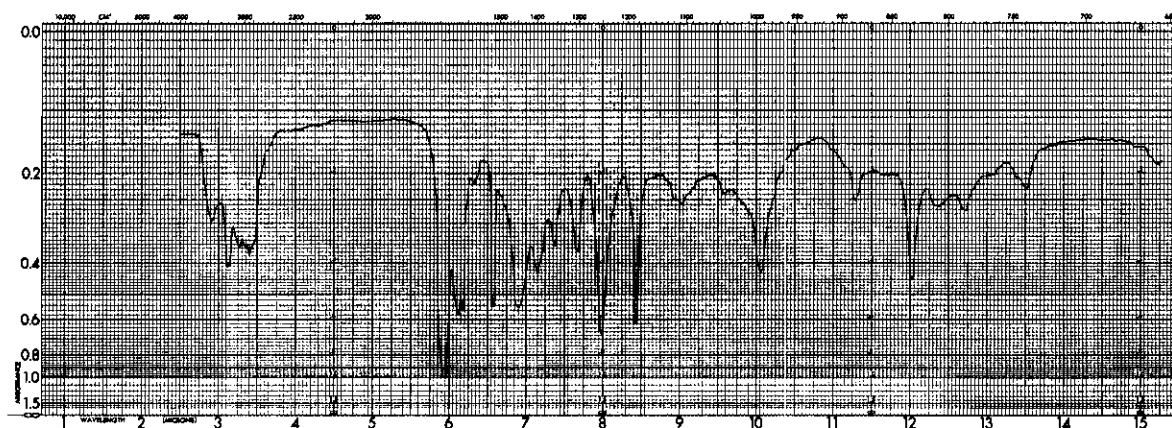


Figure 1. Infrared Spectrum of 3-(p-Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine

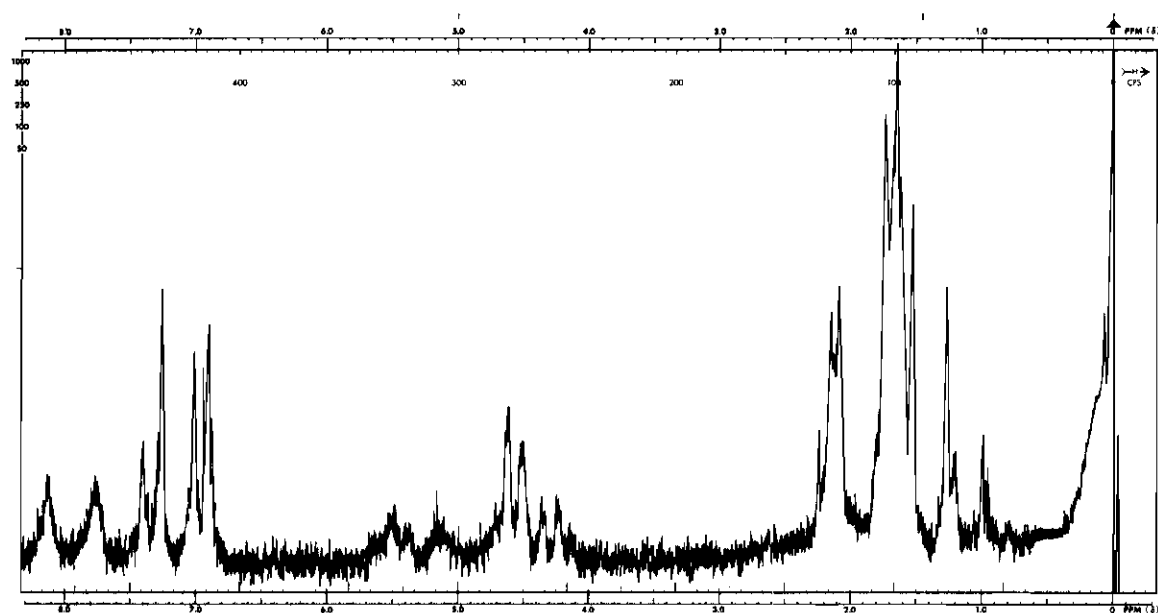


Figure 2. N.M.R. Spectrum of 3-(p-Geranyloxybenzylidene)-6-methyl-2,5-diketopiperazine



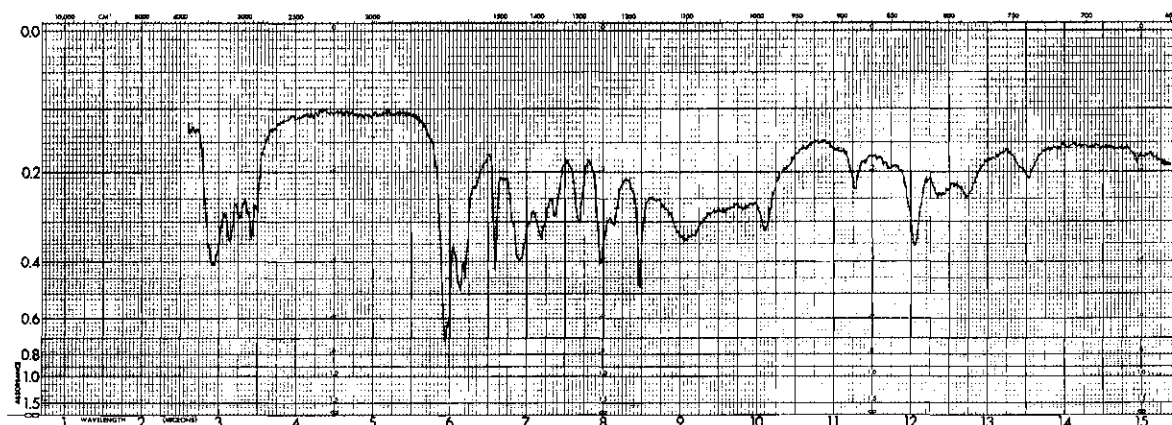


Figure 3. Infrared Spectrum of 3-[p-(trans-Ethylidene-6-methyl-5-heptenoxy)benzylidene]-6-methyl-2,5-diketopiperazine

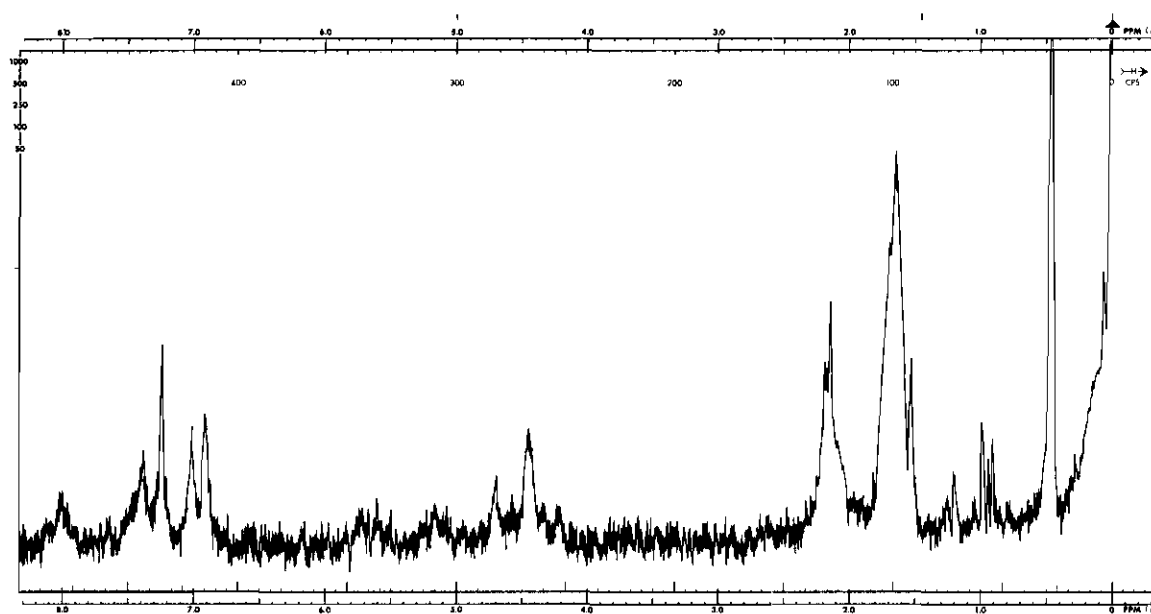


Figure 4. N.M.R. Spectrum of 3-[p-(trans-Ethylidene-6-methyl-5-heptenoxy)benzylidene]-6-methyl-2,5-diketopiperazine

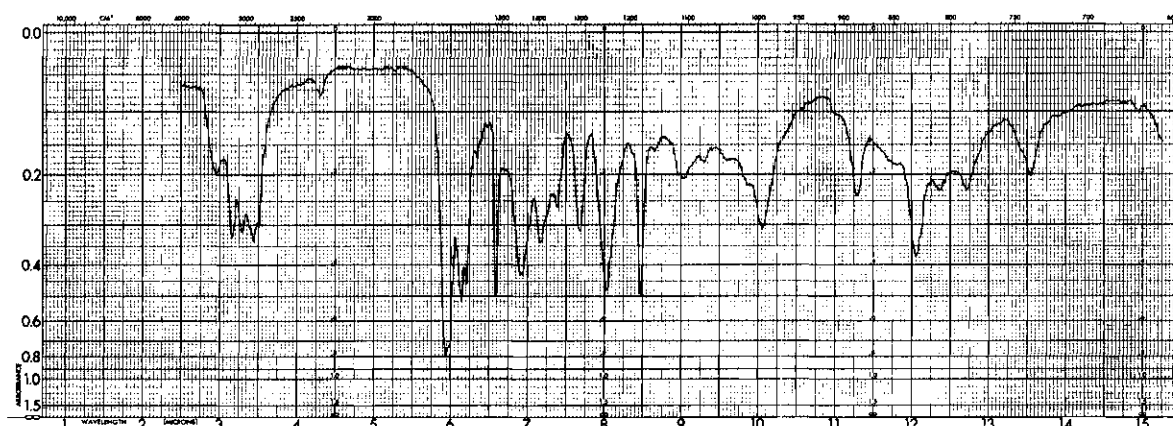


Figure 5. Infrared Spectrum of 3-[p-(2-cis-Ethylidene-6-methyl-5-heptenoxyl)benzylidene]-6-methyl-2,5-diketopiperazine

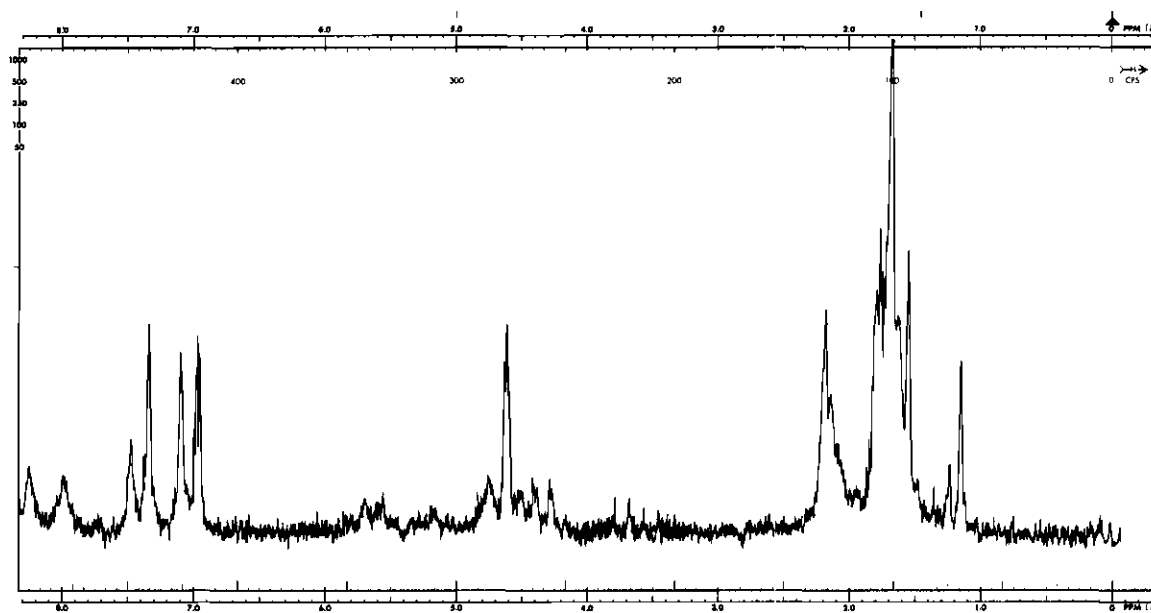


Figure 6. N.M.R. Spectrum of 3-[p-(2-cis-Ethylidene-6-methyl-5-heptenoxyl)benzylidene]-6-methyl-2,5-diketopiperazine

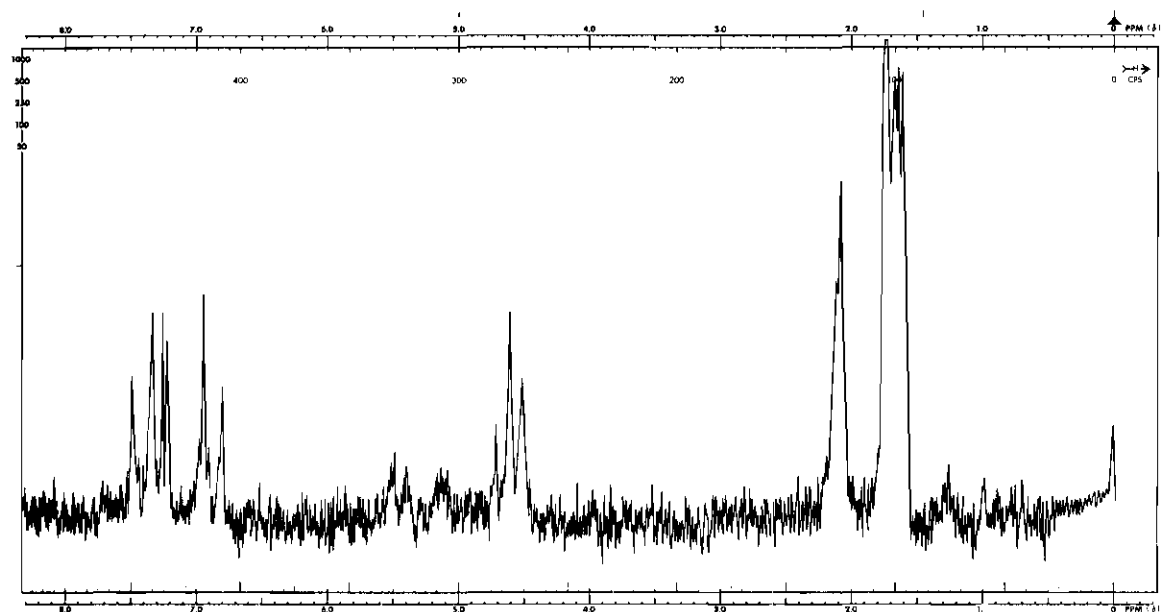


Figure 7. N.M.R. Spectrum of Mycelianamide

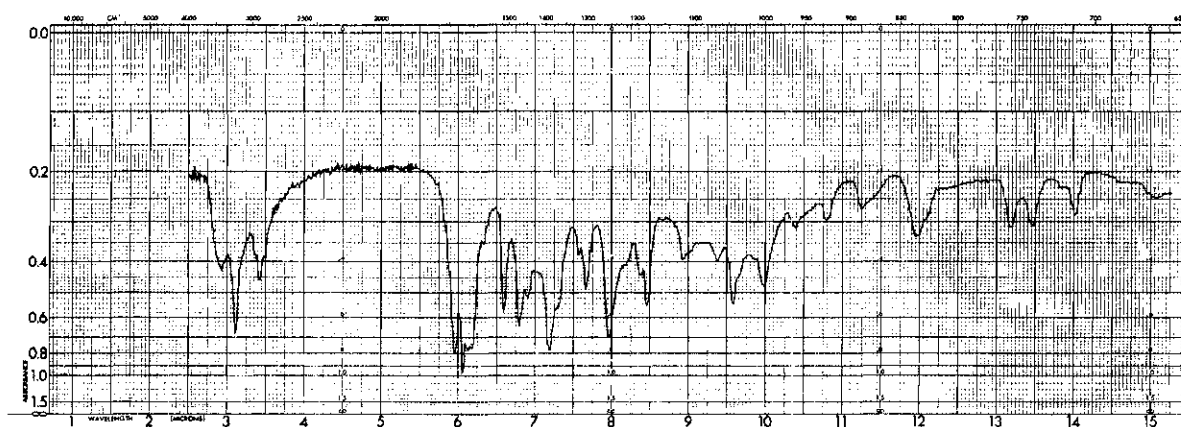


Figure 8. Infrared Spectrum of Mycelianamide

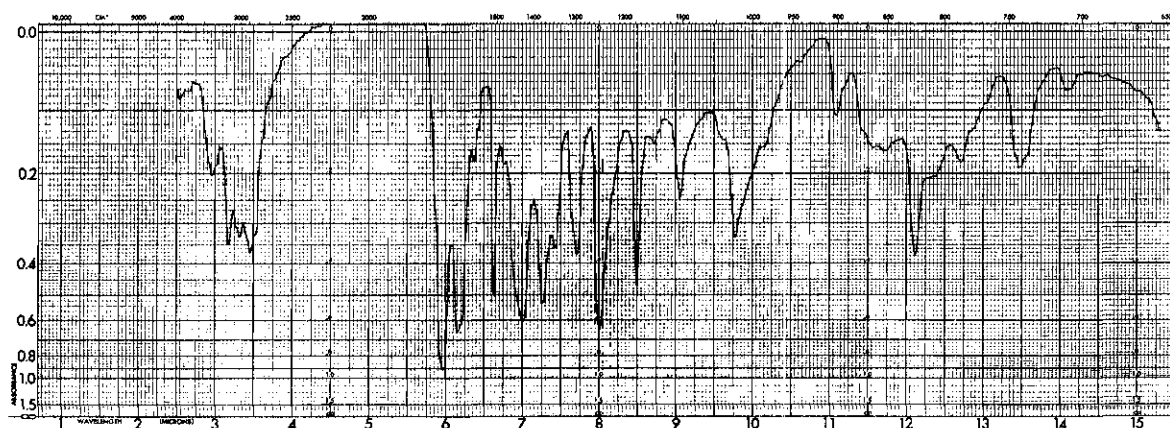


Figure 9. Infrared Spectrum of the Reduction Product of Mycelianamide

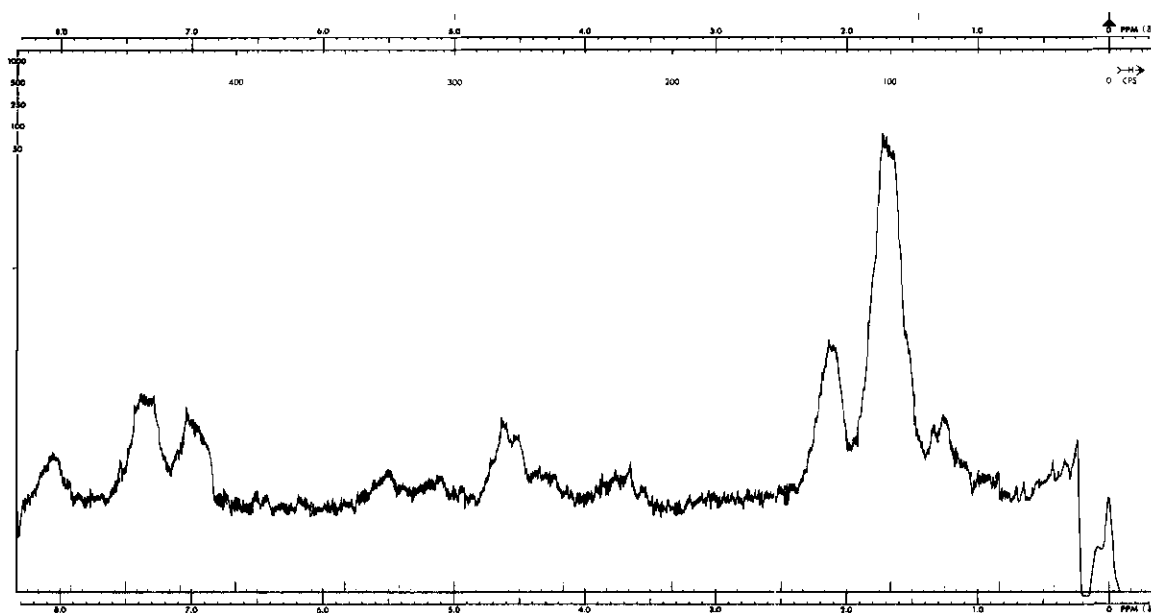


Figure 10. N.M.R. Spectrum of the Reduction Product of Mycelianamide

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\*Abbreviations used here follow the form found in Chemical Abstracts.

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## VITA

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